DOCTOR OF PHILOSOPHY

The fate and impact of groundwater nitrogen contamination on dune slack ecology

Rhymes, Jennifer

Award date:
2015

Awarding institution:
Bangor University

The Fate and Impact of Groundwater Nitrogen Contamination on Dune Slack Ecology

A thesis submitted to Bangor University by:

Jennifer Rhymes

In candidature for the degree of:

Philosophiae Doctor

May 2015

School of Biological Sciences
Bangor University

Abstract

Dune slacks are seasonal wetlands, high in biodiversity, which experience considerable within-year and between-year variations in water table. They are subject to many pressures including climate change, land use change and eutrophication. Despite their biological importance and the threats facing them, the hydrological and nutrient parameters that influence their soil properties and biodiversity are poorly understood and there have been no empirical studies to date testing for biological effects in dune systems resulting from groundwater nutrients at low concentrations, along with the interaction of lowered water tables. In this study we examined the impact of groundwater nutrients on water chemistry, soil chemistry, soil enzyme activity, greenhouse gas fluxes, vegetation composition and plant tissue chemistry. Experimental work was either; 1) undertaken at a designated sand dune site protected for its nature conservation interests (Aberffraw, North Wales, Anglesey, UK) or 2) a mesocosm experiment and laboratory assays using material collected from this site. Our findings identified that dune slack habitats are vulnerable to nitrogen groundwater contamination from concentrations as low as 0.2 mg l^{-1}, a concentration described as 'no cause for concern' on dune slack habitats by the Ecohydrological guidelines for wet dune habitats. These concentrations were seen to increase soil nitrate concentrations and increase nitrophilous species whilst decreasing basipholous species. Our study also suggests that these impacts are likely to be intensified by climate change or water abstraction, as lowered water tables decreased denitrification rates which subsequently increased soil nitrogen concentrations. The uptake, processing and accumulation of nitrogen within sand dune systems increased from groundwater nitrogen contamination, where soil nitrogen concentrations, plant tissue nitrogen content and denitrification rates increased. We also demonstrate a combination of chemical, microbial and fluorescent techniques to help identify potential nutrient contamination sources and pathways, which in turn, can inform appropriate management plans. These findings highlight the necessity to consider groundwater nutrient inputs in addition to atmospheric nitrogen inputs in wetland systems when considering nutrient impacts.

Acknowledgements

Firstly, I would like to thank my PhD supervisors Dr. Laurence Jones (Centre for Ecology and Hydrology) and Dr. Nathalie Fenner (School of Biological Sciences, Bangor University) for their relentless support, expertise and advice throughout this project, and not to forget teaching me the correct use of “effect” and “affect”. I would also like to extend my thanks to Tim Jones and Steve Hughes for their time and patience whilst carrying out analytical techniques within the laboratory.

I would like to acknowledge the funding provided by the European Social Fund (ESF) through the European Union’s Convergence programme administered by the Welsh Government. I am also grateful for the financial and botanical advice from co-sponsors Ecological Surveys, Bangor, and in particular Hilary Wallace. I also owe a great debt of gratitude to the British Geological Society, Wallingford for the use of their laboratory equipment.

Finally, I am grateful to my mum and dad, for their encouragement and financial help over the past three years and Leo Clarke, for his support and making sure I stay sane and keep smiling.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

BG	β-glucosidase
CFU	Colony Forming Units
DIC	Dissolved Inorganic Carbon
DIN	Dissolved Inorganic Nitrogen
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
DON	Dissolved Organic Nitrogen
FA	Fulvic-like
FIB	Faecal indicator bacteria
GWDTE	Groundwater Dependant Terrestrial Ecosystem
N	Nitrogen
NAG	N-acetyl-β-glucosaminidase
POX	Phenol oxidase
SAC	Special Area of Conservation
TC	Total Carbon
TCS	Total Coliforms
TIC	Total Inorganic Carbon
TN	Total Nitrogen
TRP	Tryptophan-like

CHAPTER 1: **Introduction**

1.1 Introduction

Sand dune systems have a global distribution (Martinez et al., 2004) and support a high biodiversity, including many threatened plant, insect and other animal species (Rhind and Jones, 2009, Howe et al., 2010). The low-lying depressions within these sand dune systems are dune slack habitats, which usually experience seasonal groundwater fluctuations and flooding. They are rich in species that are unique to sand dune systems or are associated with other threatened wetland habitats, such as lowland calcareous habitats and nutrient-poor, base-rich wetland fens. Within Europe these are recognised at an international level through the designation of Natura 2000 nature protection areas, made up of Special Area of Conservation (SACs) sites under the European Habitats Directive (92/43/EEC) Annex I humid dune slack habitat (code 2190). These support Annex 2 species in the Habitats Directive which include: *Liparis loeselii, Gentianella anglica, Petalophyllum ralfsii, Vertigo angustior* and *Triturus cristatus.* Sand dune systems have undergone considerable change globally in the last century (Martinez et al., 2004) including; afforestation (Stratford et al., 2007), inappropriate grazing management (Plassmann et al., 2010), nitrogen pollution (Jones et al., 2013), crashing rabbit populations (Provoost et al., 2011) and water table lowering from either climate change (Clarke and Ayutthaya, 2010) or groundwater abstraction (Provoost et al., 2011). As a result of these multiple pressures, dune slacks are a threatened habitat and, with increasing vulnerability from multiple threats, there is potential for future species and habitat loss.

1.2 Dune slack vegetation

Dune slacks are a priority habitat for rare orchids (*Liparis loeselii, Dactylorhiza praetermissa and Dactylorhiza purpurella*), liverworts (*Petalophyllum ralfsii*), stoneworts and *Bryum* mosses. Many of these species are intolerant of competition and are therefore confined to succesionally young vegetation communities (Rhind and Jones, 1999, Sival et al., 1998). Within the United Kingdom there are five classified dune slack vegetation communities identified by the National Vegetation Classification (NVC) system (Rodwell et al., 2000) with a number of sub-communities. These communities are summarised in Table 1.1 and differ in water table depths, age and pH characteristics. The vegetation of dune slacks is predominantly controlled by water table depth, seasonal water table fluctuations and water chemistry (Curreli et al., 2013, Grootjans et al., 1996, Lammerts and Grootjans, 1997, Willis et al., 1959).

Table 1.1 Summary of NVC communities and sub-communities identified by Curreli et al. (2013)

NVC code	**Name**	**Description**
SD 13	*Sagina nodosa-Bryum pseudotriquetrus* community	Early successional stage, rich in bryophytes and liverworts, usually with bare sand. Fairly drought tolerant.
SD 14	*Salix repens-Calliergon cuspidatum stellatum* community	Frequently species rich and associated with persistently humid soils and base-rich groundwater.
SD 14b	*Rubus caesius-Gallium palustre* subcommunity	Some of its constant species (*Ranunculus flammula, Carex nigra*) can indicate tolerance to very wet periods.
SD 14c	*Bryum Psuedotriquetrus-Aneura pinguis* subcommunity	Young successional stage, mosses have sparse cover, heliophilous and pioneer species can be present.
SD 14d	*Festuca rubra* subcommunity	Characteristic of drier substrates, it can be an intermediate stage towards grass encroachment.
SD 15b	*Salix repens-Calliergon cuspidatum* community *Equisetum variegatum* subcommunity	Late successional stage, generally species poor. Less dependent on base-richness of water, but strongly related with flooding.
SD 16	*Salix repens-Holcus lanatus* community	Late successional stage in dry slacks. Dominated by fescue and other grasses, forbs are still indicative of calcicolous substrate.
SD 17	*Potentilla anserina-Carex nigra* community	Species composition reflects damp habitat, recalling fen meadows. Forb-rich, with a sparse shrub cover.

1.3 Threats and their biological impacts on dune slacks

1.3.1 Nitrogen contamination

Humans have significantly increased the global availability and mobility of nitrogen over the past two centuries (Galloway and Cowling, 2002). Sources of nitrogen pollution from anthropogenic activities include point and non-point sources (see Table 1.2 for examples). Many of these sources cause nitrogen pollution in the form of inorganic nitrogen (nitrate, nitrite and ammonium ions), these can then enter aquatic ecosystems via multiple pathways such as surface run off, streams and groundwater and tracing these sources is often challenging (Withers et al., 2009).The impacts of nitrogen on river, stream, groundwater and groundwater dependant terrestrial ecosystems (GWDTEs) are recognised under the EU Water Framework Directive. It is therefore necessary to quantify groundwater nitrogen concentrations at which biological impacts occur within GWDTEs and to determine the source pollutants adequately. With regards to inputs from agricultural practices and fertilisers in particular, estimates suggest that there will be a 50% increase in global fertiliser

usage by 2050 (Alexandratos and Bruinsma, 2012) and that this will increase eutrophication events. Generally, these nonpoint sources are more difficult to manage than point sources because they are much larger (Howarth et al., 2002), and therefore they pose serious threats to sand dune systems located near to agricultural areas.

Nitrogen sourced from atmospheric deposition has significantly increased from its pre-industrial levels of 2-6 kg N ha^{-1} yr^{-1} (Fowler et al., 2005) and, as a result, most atmospheric depositions within sand dune systems across Europe are seen to exceed the defined dune slack specific critical nitrogen load of 10-15 kg N ha^{-1} yr^{-1} (Bobbink and Hettelingh, 2010). Whilst the effects of atmospheric deposition on dry dune habitats have received recent attention (Plassmann et al., 2009, Remke et al., 2009, Jones et al., 2013), comparatively little attention has been given to dune slack habitats and impacts from other sources of nutrients. A collation of dune groundwater chemistry data suggested that values >1 mg l^{-1} of dissolved inorganic nitrogen (DIN) within the groundwater indicated probable nutrient contamination (Davy et al., 2010). A global assessment of aquatic ecosystems concluded that concentrations above 0.5 – 1.0 mg l^{-1} of total nitrogen (TN) could lead to eutrophication (Camargo and Alonso, 2006). Conversely the United Kingdom Technical Advisory Group (UKTAG, 2014), who are responsible for providing advice on technical aspects of the Water Framework Directive, suggest threshold values as high as 3 mg l^{-1} of nitrogen (N) for dune slack habitats. However, the biological effects of groundwater nitrogen contamination and the concentrations at which biological effects occur have not yet been investigated within dune slack habitats.

Table 1.2 Major anthropogenic sources of inorganic nitrogen into aquatic ecosystems. Extracted from Camargo and Alonso (2006).

Point Sources
-Wastewaters from livestock (cattle, pigs, chickens) farming
-N releases from aquaculture (fish, prawns, shrimps) operations
-Municipal sewage effluents (including effluents from sewage treatment plants that are not performing tertiary treatments)
-Industrial wastewater effluents
-Runoff and infiltration from waste disposal sites
-Runoff from operational mines, oil fields, and unsewered industrial sites
-Overflows of combined storm and sanitary sewers
Nonpoint sources
-Widespread cultivation of N_2-fixing crop species, and the subsequent N mobilisation among terrestrial, aquatic and atmospheric realms
-Use of animal manure and inorganic N fertilisers, and the subsequent runoff from agriculture
-Runoff from burned forests and grasslands
-Runoff from N saturated forests and grasslands
-Urban runoff from unsewered and sewered areas
-Septic leachate and runoff from septic tanks
-Runoff from construction sites and abandoned mines
-N loadings to ground waters and, subsequently, to receiving surface waters (rivers, lakes, estuaries, coastal zones)
-Emissions to the atmosphere of reduced (from volatilisation of manure and fertilisers) and oxidised (from combustion of fossil fuels) N compounds, and the subsequent atmospheric (wet and dry) deposition over surface waters
-Other activities that can mobilise nitrogen (from long-term storage pools) such as biomass burning, land clearing, and conversion and wetland draining

Young dune slacks are base rich and low nutrient habitats and are considered to be at threat from eutrophication (Bobbink et al., 2003). The impacts from atmospheric nitrogen deposition on sand dune systems are better understood (Bobbink et al., 2010, Krupa, 2003) than any other sources of nitrogen. Previous studies have shown increases in biomass (Van den Berg et al., 2005, Plassmann et al., 2009), and, in some cases, these impacts have been observed within dry dune habitats subject to nitrogen depositions lower than dry dune habitat critical loads of 11-14 kg N ha^{-1} yr^{-1} (Jones et al., 2013). Increased nitrogen plant tissue content as a result of increased nitrogen uptake has been observed within bryophytes (Plassmann et al., 2009) and various dune grassland species (Jones et al., 2013) following the addition of nitrogen. Other nitrogen addition studies found dune slacks to

become dominated by *Agrostis stolonifera* (Willis, 1963) and increased biomass (Olff et al., 1993), however these two studies applied unrealistically high nitrogen concentrations. Overall, studies that have investigated the effects of nitrogen within sand dune systems suggest that nitrogen inputs accelerate succession, increase biomass, and in turn cause losses of bryophytes and other species associated with early succession (Van den Berg et al., 2005, Veer, 1997).

Nutrients return to the soil surface as litter fall (Berendse et al., 1998) and within dune slacks with nitrogen contamination issues these nutrients are likely to be higher, as a result of increases in biomass (Van den Berg et al., 2005) and increased plant tissue nitrogen (Jones et al., 2013). This in turn thickens the organic layer, increases soil development and soil nutrient availability and consequently affects soil biological processes (Emmett, 2007, Jones et al., 2008). Increased soil nitrate availability can also alter microbial communities (Peacock et al., 2001) and increase denitrification activity (Merrill and Zak, 1992), with subsequent release of nitrogen back into the atmosphere in gaseous form (Myrold, 1998). Nonetheless, these soil processes have not been well studied within dune slack soils.

1.3.2 Climate change

An emerging threat to dune slack habitats is that of climate change, with the potential of altering dune slack environmental conditions. Water table depth is largely affected by groundwater recharge, which is mediated by the balance between rainfall and evapotranspiration rates. With a global increase in temperatures and altered rainfall patterns (Allan and Soden, 2008), the balance between rainfall and evapotranspiration within dune slacks will be affected. A recent study suggests that groundwater levels are likely to drop within dune slacks located in the North West of England as a result of climate change (Clarke and Ayutthaya, 2010) and that this may alter water table depths by up to 100 cm by 2080 (Fig 1.1). The lowering of water tables, however, can also occur as a result of water abstraction practices to supply drinking water. This practice frequently occurs within some European countries such as the Netherlands (Grootjans et al., 1998).

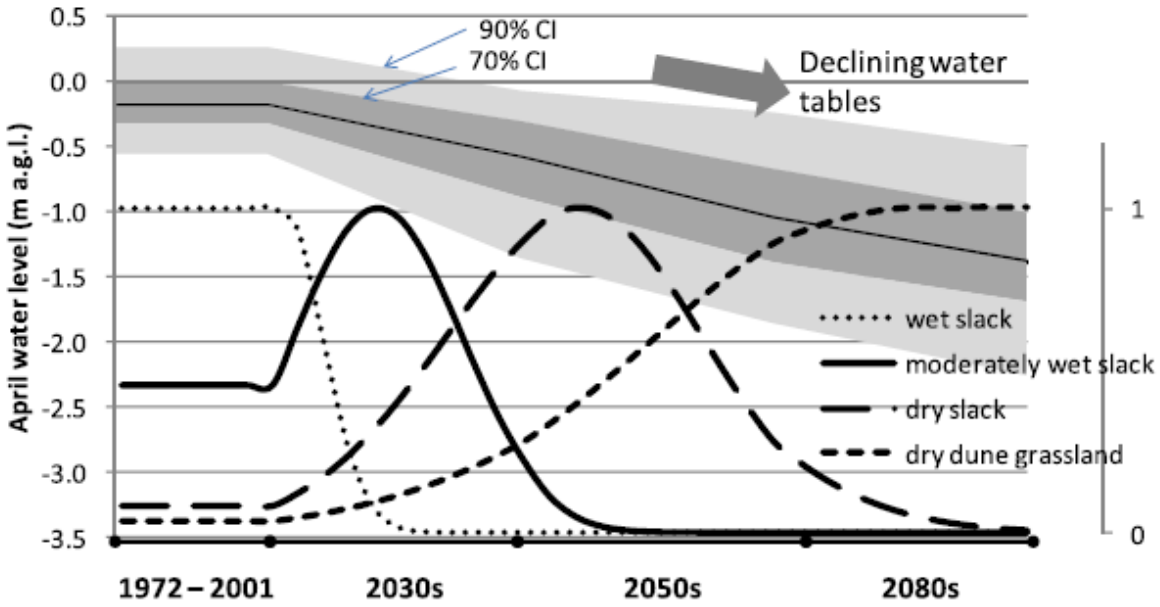


Fig 1.1 Frequency distribution curves of median water level for different slack types (data rescaled on 2nd y axis to max=1) plotted against predicted changes in April water levels (meters above ground level, 1st y axis) of a currently wet dune slack (SD15) at Ainsdale in northwest England. Predicted April water levels show central estimate (thin black line), 75% confidence interval (dark grey zone) and 90% confidence interval (light grey zone). Extracted from Curreli et al. (2013).

The hydrological characteristics of the NVC dune slack communities (Table 1.1.) are well recognised (Rodwell et al., 2000, Davy et al., 2010) and a recent study by Curreli et al. (2013) has quantified the eco-hydrological tolerance of dune slack communities to dropping water table depths. The results of this study showed that the wettest communities were only separated from the driest by a 40 cm difference in water table depth, highlighting the potential for wet dune slack vegetation assemblages to shift to dry grassland characteristics.

Dune slacks experience seasonal fluctuation in water table depth, with periods of flooding within the winter months (van der Laan, 1979) leading to reduced mineralisation of organic matter and increased denitrification rates , thus conserving the characteristically low nutrient status (Berendse et al., 1998). Such soil processes are essential in regulating the impacts of nitrogen within slack habitats that are vulnerable to nitrogen contamination. The threat of lowered water table depths could therefore alter these essential soil processes and could also intensify the impacts from groundwater nitrogen contamination.

1.4 Techniques to identify soil processes

1.4.1 Extracellular enzyme techniques

One technique for measuring processes and rates of nutrient and carbon cycling within ecosystems is the measurement of soil extracellular enzymatic activity, which is frequently used within wetland science. Such techniques allow for the understanding of microbial activity under environmental pressures, since microbial communities are the dominant producers of extracellular enzymes over plant roots (Kang and Freeman, 1999). To our knowledge, these techniques have not yet been carried out within dune slack habitats, however they have been employed for multiple soil types where their responses to environmental pressures are well documented (Henry, 2012).

The hydrolase enzyme N-acetyl-β-glucosaminidase (NAG) is responsible for the breakdown of chitin, an essential process in nitrogen cycling (Kang et al., 2005), and β-glucosidase (BG) for the degradation of cellulose, providing labile carbon to soil microbes (Deng, 2011). Phenol oxidase enzyme (POX) degrades phenolic material (McLatchey and Reddy, 1998). Although this is not involved with nitrogen cycling directly, the accumulation of phenolics from low POX activity can mediate the activity of hydrolase enzymes, such as NAG (Freeman et al., 2001). The measurement of POX therefore helps the interpretation of NAG responses to nitrogen contamination.

The anthropogenic alteration of nitrogen availability from increased agricultural pressures and fertiliser usage, can essentially increase nutrient availability and increase the thickness of soil organic matter (Emmett, 2007). In turn, this can alter microbial communities (Peacock et al., 2001) and alter decomposition and nitrogen cycling rates. Within wetlands, extracellular enzyme activity is constrained by soil conditions. Certainly, within peatlands the acidic, cool and anaerobic conditions are thought to constrain POX activity (Freeman et al., 2001, Tahvanainen and Haraguchi, 2013). Subsequently, soil phenolics increase which in turn, inhibit hydrolase enzymes and reduce decomposition rates (Freeman et al., 1990, Wetzel, 1992). In response to lowering water tables, whereby soil moisture decreases, POX is likely to be stimulated, lowering phenolic concentrations and subsequently increasing hydrolase enzyme activity (Freeman et al., 2001). As these techniques have not been carried out within dune slack soils previously, investigations are required to determine whether similar controls are true within these systems in response to both nitrogen contamination and climate change.

1.4.2 Greenhouse gas fluxes

Greenhouse gases (GHG) are responsible for enhancing global warming; these include CO_2, CH_4 and N_2O. The measurement of GHG can indicate specific microbial activity and microbiological processes within soils and fluxes between the atmosphere and soil/vegetation are shown in Fig 1.2. Carbon dioxide is produced by autotrophic and heterotrophic respiration, whilst it is consumed by vegetation during photosynthesis and microbial autotrophy (Blais et al., 2005). Methane is mostly produced by methanogens under anaerobic conditions (Chaban et al., 2006); however, it can also be consumed by anaerobic microbes (i.e. methanotrophs) within anaerobic conditions. Nitrous oxide is produced by at least three microbial processes; 1) nitrification 2) denitrification and 3) assimilatory nitrate reduction (Dalal and Allen, 2008). Microbial denitrification is the process by which soil nitrate is reduced to gaseous nitrogen products N_2, N_2O and NO by microbial processes (Knowles, 1982), the measurement of N_2O within wetland studies is often used as an indicator of soil denitrification (Bernot et al., 2003, DeLaune and Jugsujinda, 2003). N_2O can, however, be consumed by soil and vegetation sorption during anaerobic carbon mineralisation (Chapuis-Lardy et al., 2007).

In response to increased soil nitrogen availability denitrification rates tend to increase (Merrill and Zak, 1992) where water content allows. This results in increased N_2O emissions as increased soil nitrate inhibits the reduction of N_2O to N_2 (Blackmer and Bremner, 1978). In some cases, however, denitrification rates do not change with increased nitrogen availability as the process can be limited by soil carbon stocks (Weier et al., 1993) and temperature. In drier soil conditions decomposition rates increase with increased autotrophic and heterotrophic microbial respiration. Within wetter soils anaerobic conditions persist, resulting in increased methanogenesis and therefore increased soil methane emissions. There is limited understanding of these processes within dune slack soils at present, though particularly with regard to specific processes and their responses to emerging groundwater nitrogen contamination and climate change.

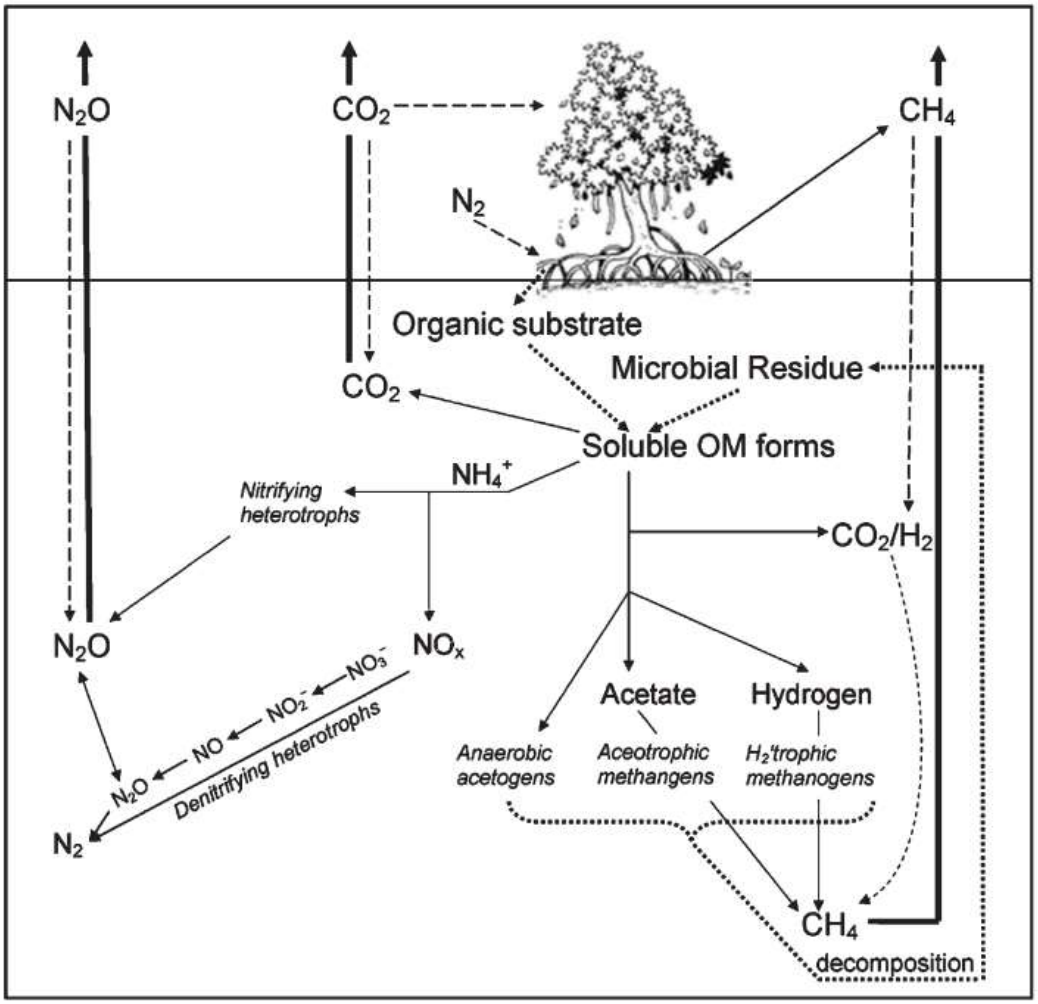


Fig 1.2 Flow diagram of GHG production and consumption in soils extracted from Dalal and Allen (2008). Additional nitrogen from atmospheric deposition, fertilisers and plant based emissions are not included in this diagram.

1.5 Techniques to source and trace groundwater nutrients

Nitrogen diffuse inputs have largely been characterised by using total nitrogen as a proxy (e.g. Vadas et al., 2007). More recently however, inputs can be separately identified as inputs from either livestock, humans, inorganic inputs or a combination by measuring pathogenic micro-organisms (Kay et al., 2008), such as faecal indicator bacteria, or by characterising dissolved organic matter (DOM) by natural fluorescence (Hudson et al., 2007). These methods can allow for a more detailed description of the inputs and pathways of nitrogen contamination within most ecosystems.

1.5.1 Faecal indicator bacteria

The enumeration of faecal indicator bacteria detects and estimates the contribution of faecal contamination within waters, and may indicate potential health risks. Generally, these techniques are used to measure drinking water quality (WHO, 2006) and water quality within the revised Bathing Waters Directive (European Community, 2006). With regards to drinking water some countries (eg. Netherlands) extract groundwater from sand dune systems to supply drinking water, indicating the importance of understanding the survival and pathways of faecal bacteria within these systems (Smeets et al., 2009).

The most commonly tested faecal indicator bacteria include total coliforms (TC) and sub group *Escherichia coli* (*E. coli*). These are generally considered to be specific to faecal contamination, although TCs also include other genera such as *Klebisella* and *Citrobacter.* These genera are not necessarily of faecal origin however and can originate from other organic sources such as decaying plant materials and soils (WHO, 2006). The main sources of *E. coli* include septic tanks, sewer lines, wastewater treatment plants, manure spreading, livestock and wildlife. The enumeration of *E. coli* can therefore identify potential diffuse sources of faecal contamination, which is necessary to aid the prevention of future eutrophication events. *E. coli* can however grow and maintain populations in the environment if the conditions are suitable (Byappanahalli and Fujioka, 2004), and therefore must be considered when identifying sources. The relationship between the transport time and distance of bacteria to eventually reach groundwaters or streams is dependent on the rate at which bacteria are released from manure, the presence of preferential pathways within the soil and the depth of the groundwater (Abuashour et al., 1994, Unc and Goss, 2003).

1.5.2 Natural fluorescence spectroscopy

Excitation emission matrix fluorescence spectroscopy (EEMS) is used to trace DOM from agricultural sources (Baker, 2002, Old et al., 2012). This technique is quick, cheap and sensitive enough to characterise fulvic-like, humic-like and protein-like substances (tryptophan-like and tyrosine like) within the DOM (Fig 1.3). This essentially characterises and quantifies the extent of contamination by effluents from different sources within water samples (Hudson et al., 2007). Fulvic-like and humic-like substances are derived from the breakdown of plant material (Stedmon et al., 2003) whereas large inputs of tryptophan-like substances are associated with readily biodegradable material from faecal origin such as sewage and farm waste slurry (e.g. Baker, 2001). Agricultural diffuse sources such as animal waste are characterised by high protein-like fluorescence, with very high ratios of tryptophan-like to fulvic/humic-like fluorescence compared to stream waters (Baker,

2002). These ratios are also sensitive enough to characterise inputs from different livestock animals such as pigs and sheep (Baker, 2002). The use of both faecal indicator bacteria and natural fluorescence will therefore prove extremely useful when attempting to identify nitrogen contamination sources and pathways within sand dune systems subject to groundwater contamination.

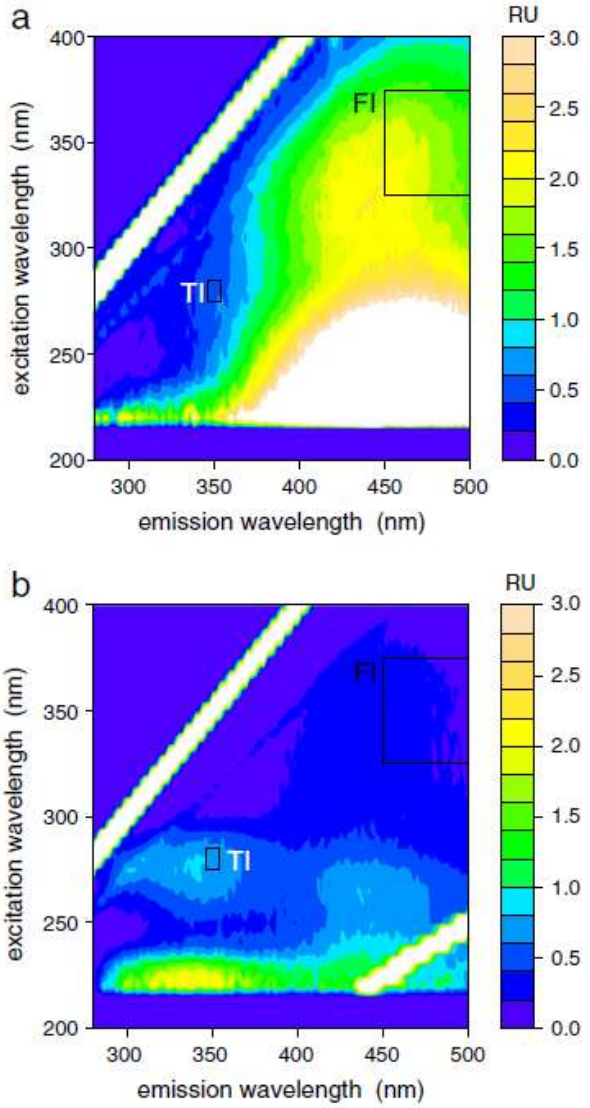


Fig 1.3 Example of corrected excitation-emission matrices in Raman units: a) Grassland water sample; b) diluted farmyard water sample. Boxes show areas used to calculate the tryptophan (TI) and fulvic/humic (FI) indices. Extracted from Old et al. (2012).

1.6 Scope of study

Both field and laboratory experimental work presented in this thesis was either; 1) undertaken at a designated sand dune site protected for its nature conservation interests (Aberffraw, North Wales,

Anglesey, UK) or 2) using material collected from this site. The threat to sand dune systems from increasing groundwater nitrogen availability and climate change is poorly understood. Previous studies that have investigated impacts from atmospheric nitrogen sources suggest that such threats will cause detrimental impacts to sand dune sites across Europe, with the potential for habitat and species losses protected under EU legislation. No studies to date however have investigated the impacts of groundwater nitrogen contamination and the interaction with the emerging threat from climate change. This thesis was motivated by a desire to better understand the response of vegetation and soil processes to these threats. In addition, this project investigated the use of multiple analytical techniques to identify potential nitrogen sources within the groundwater, with a view to improve conservation techniques. The main aims of this thesis are as follows:

- To examine the impacts of nitrogen groundwater contamination on dune slack vegetation and soil chemistry, and at what concentrations such impacts occur.
- To investigate the impacts from the interaction of both climate change and groundwater nitrogen contamination on soil processes and the fate of that nitrogen.
- To utilise a combination of inorganic chemistry analytical techniques to evaluate the potential sources and pathways of groundwater nutrients and contamination to sand dune sites.

1.7 Thesis structure

Chapter 2 examines *in situ* groundwater nitrogen impacts on dune slack habitat through water and soil chemistry integrated with a botanical survey. Measurements were taken along a gradient of nutrient impacts from known sources, whilst controlling for differences in water table depths. This chapter aims to quantify groundwater nitrogen concentrations at which impacts occur.

Chapter 3 explored the use of a combination of inorganic chemistry, DOM fluorescence and culturable *E. coli* and TC counts to evaluate the potential sources and pathways of nutrients and contamination *in situ* within a sand dune site known to be affected by nutrients from surrounding agricultural land. This chapter aims to assess the efficacy of such techniques in combination, to aid and inform wetland management.

Chapter 4 uses a combination of mesocosm and laboratory microcosm experiments to identify the impact of groundwater nitrogen contamination alone, as well as the interaction between groundwater contamination and climate change with a focus on soil denitrification, decomposition

and biogeochemistry. Measurements include soil chemistry, extracellular enzyme activity and greenhouse gas fluxes.

Chapter 5 presents results from a groundwater nitrogen and water table depth mesocosm manipulation experiment designed to investigate the interactions between groundwater nitrogen contamination and climate change on vegetation nitrogen uptake and species success. The investigation also included a water and nitrogen budget to better understand the fate of nitrogen within dune slack habitats.

The chapters in this thesis have been written as a series of manuscripts prepared for publication in peer reviewed scientific journals, with two having recently been published (Chapters 2 and 3). The published articles of these chapters are presented in appendices 6.2 and 6.3. Details of published and submitted papers are given below. The contributions of co-authors are detailed at the beginning of each chapter.

1.8 Published articles

Chapter 1: Rhymes, J., Wallace, H., Fenner, N. & Jones, L. 2014. Evidence for sensitivity of dune wetlands to groundwater nutrients. *Science of the Total Environment,* 490C**,** 106-113.

Chapter 2: Rhymes, J., Jones, L., Fenner, N., Lapworth, D., White, D., McDonald, J., Perkins, T. 2015. Chemical, microbial and fluorescence techniques to understand contaminant sources and pathways to wetlands in a conservation site. *Science of the Total Environment, 511,703-710*

1.9 Conference presentations

Chapter 1: This chapter was orally presented at The Sand Dune and Shingle conference 9^{th}-11^{th} September, 2013 and as a poster at The Joint Aquatic Science Meeting 18^{th}-23^{rd} May, 2014 (See Appendix III).

1.10 References

ABUASHOUR, J., JOY, D. M., LEE, H., WHITELEY, H. R. & ZELIN, S. 1994. Transport of microorganisms through Soil. *Water Air and Soil Pollution,* 75**,** 141-158.

ALEXANDRATOS, N. & BRUINSMA, J. 2012. World agriculture towards 2030/2050: the 2012 revision. ESA Working paper Rome, FAO.

ALLAN, R. P. & SODEN, B. J. 2008. Atmospheric warming and the amplification of precipitation extremes. *Science,* 321**,** 1481-1484.

BAKER, A. 2001. Fluorescence excitation-emission matrix characterization of some sewage-impacted rivers. *Environmental Science & Technology,* 35**,** 948-953.

BAKER, A. 2002. Fluorescence properties of some farm wastes: implications for water quality monitoring. *Water Research,* 36**,** 189-195.

BERENDSE, F., LAMMERTS, E. & OLFF, H. 1998. Soil organic matter accumulation and its implications for nitrogen mineralization and plant species composition during succession in coastal dune slacks. *Plant Ecology,* 137**,** 71-78.

BERNOT, M. J., DODDS, W. K., GARDNER, W. S., MCCARTHY, M. J., SOBOLEV, D. & TANK, J. L. 2003. Comparing denitrification estimates for a Texas estuary by using acetylene inhibition and membrane inlet mass spectrometry. *Applied and Environmental Microbiology,* 69**,** 5950-5956.

BLACKMER, A. & BREMNER, J. 1978. Inhibitory effect of nitrate on reduction of N_2O to N_2 by soil microorganisms. *Soil Biology and Biochemistry,* 10**,** 187-191.

BLAIS, A.-M., LORRAIN, S. & TREMBLAY, A. 2005. Greenhouse gas fluxes (CO_2, CH_4 and N_2O) in forests and wetlands of boreal, temperate and tropical regions. *Greenhouse Gas Emissions—Fluxes and Processes.* Springer.

BOBBINK, R., ASHMORE, M., BRAUN, S., FLÜCKIGER, W. & VAN DEN WYNGAERT, I. J. 2003. Empirical nitrogen critical loads for natural and semi-natural ecosystems: 2002 update. *Empirical critical loads for nitrogen***,** 43-170.

BOBBINK, R. & HETTELINGH, J.-P. Review and revision of empirical critical loads and dose-response relationships. Proceedings of an expert workshop, Noordwijkerhout, 2010. 23-25.

BOBBINK, R., HICKS, K., GALLOWAY, J., SPRANGER, T., ALKEMADE, R., ASHMORE, M., BUSTAMANTE, M., CINDERBY, S., DAVIDSON, E., DENTENER, F., EMMETT, B., ERISMAN, J. W., FENN, M., GILLIAM, F., NORDIN, A., PARDO, L. & DE VRIES, W. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications,* 20**,** 30-59.

BYAPPANAHALLI, M. & FUJIOKA, R. 2004. Indigenous soil bacteria and low moisture may limit but allow faecal bacteria to multiply and become a minor population in tropical soils. *Water Science and Technology,* 50**,** 27-32.

CAMARGO, J. A. & ALONSO, A. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International,* 32**,** 831-849.

CHABAN, B., NG, S. Y. & JARRELL, K. F. 2006. Archaeal habitats-from the extreme to the ordinary. *Canadian Journal of Microbiology,* 52**,** 73-116.

CHAPUIS-LARDY, L., WRAGE, N., METAY, A., CHOTTE, J. L. & BERNOUX, M. 2007. Soils, a sink for N_2O? A review. *Global Change Biology,* 13**,** 1-17.

CLARKE, D. & AYUTTHAYA, S. S. N. 2010. Predicted effects of climate change, vegetation and tree cover on dune slack habitats at Ainsdale on the Sefton Coast, UK. *Journal of Coastal Conservation,* 14**,** 115-125.

COMMUNITY, E. 2006. Directive 2006/7/EC of the European Parliament and of the Council of 15 February (2006) concerning the management of bathing water quality and repealing Directive 76/160/EEC. Official Journal of the European Union, 4.3.2006, L64/37.

CURRELI, A., WALLACE, H., FREEMAN, C., HOLLINGHAM, M., STRATFORD, C., JOHNSON, H. & JONES, L. 2013. Eco-hydrological requirements of dune slack vegetation and the implications of climate change. *Science of the Total Environment,* 443**,** 910-919.

DALAL, R. C. & ALLEN, D. E. 2008. TURNER REVIEW No. 18. Greenhouse gas fluxes from natural ecosystems. *Australian Journal of Botany,* 56**,** 369-407.

DAVY, A., HISCOCK, K., JONES, M., LOW, R., ROBINS, N. & STRATFORD, C. 2010. Ecohydrological guidelines for wet dune habitats, phase 2. Protecting the plant communities and rare species of dune wetland systems. *Bristol, UK: Environment Agency*.

DELAUNE, R. & JUGSUJINDA, A. 2003. Denitrification potential in a Louisiana wetland receiving diverted Mississippi River water. *Chemistry and Ecology,* 19**,** 411-418.

DENG, S., & POPOVA, I. 2011. Carbohydrate hydrolases. In: Dick RP (ed) Mehods of soil enzymology. *Soil Science Society of America, Inc., Madison,* pp 185-209.

EMMETT, B. A. 2007. Nitrogen saturation of terrestrial ecosystems: some recent findings and their implications for our conceptual framework. *Water, Air, & Soil Pollution: Focus,* 7**,** 99-109.

FOWLER, D., O'DONOGHUE, M., MULLER, J., SMITH, R., DRAGOSITS, U., SKIBA, U., SUTTON, M. & BRIMBLECOMBE, P. 2005. A chronology of nitrogen deposition in the UK between 1900 and 2000. *Water, Air, & Soil Pollution: Focus,* 4**,** 9-23.

FREEMAN, C., LOCK, M. A., MARXSEN, J. & JONES, S. E. 1990. Inhibitory effects of high molecular weight dissolved organic matter upon metabolic processes in biofilms from contrasting rivers and streams. *Freshwater Biology,* 24**,** 159-166.

FREEMAN, C., OSTLE, N. & KANG, H. 2001. An enzymic 'latch' on a global carbon store - A shortage of oxygen locks up carbon in peatlands by restraining a single enzyme. *Nature,* 409**,** 149-149.

GALLOWAY, J. N. & COWLING, E. B. 2002. Reactive nitrogen and the world: 200 years of change. *Ambio,* 31**,** 64-71.

GROOTJANS, A., ERNST, W. & STUYFZAND, P. 1998. European dune slacks: strong interactions of biology, pedogenesis and hydrology. *Trends in Ecology & Evolution,* 13**,** 96-100.

GROOTJANS, A. P., SIVAL, F. P. & STUYFZAND, P. J. 1996. Hydro-geochemical analysis of a degraded dune slack. *Vegetation,* 126**,** 27-38.

HENRY, H. A. L. 2012. Soil extracellular enzyme dynamics in a changing climate. *Soil Biology and Biochemistry,* 47**,** 53-59.

HOWARTH, R. W., SHARPLEY, A. & WALKER, D. 2002. Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. *Estuaries,* 25**,** 656-676.

HOWE, M., KNIGHT, G. & CLEE, C. 2010. The importance of coastal sand dunes for terrestrial invertebrates in Wales and the UK, with particular reference to aculeate Hymenoptera (bees, wasps & ants). *Journal of Coastal Conservation,* 14**,** 91-102.

HUDSON, N., BAKER, A. & REYNOLDS, D. 2007. Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters - A review. *River Research and Applications,* 23**,** 631-649.

JONES, L., NIZAM, M., REYNOLDS, B., BAREHAM, S. & OXLEY, E. 2013. Upwind impacts of ammonia from an intensive poultry unit. *Environmental Pollution,* 180**,** 221-228.

JONES, M. L. M., SOWERBY, A., WILLIAMS, D. L. & JONES, R. E. 2008. Factors controlling soil development in sand dunes: evidence from a coastal dune soil chronosequence. *Plant and Soil,* 307**,** 219-234.

KANG, H. & FREEMAN, C. 1999. Phosphatase and arylsulphatase activities in wetland soils: annual variation and controlling factors. *Soil Biology and Biochemistry,* 31**,** 449-454.

KANG, H., FREEMAN, C. & CHUN, J. 2005. N-Acetylglucosaminidase activities in wetlands: a global survey. *Hydrobiologia,* 532**,** 103-110.

KAY, D., CROWTHER, J., FEWTRELL, L., FRANCIS, C. A., HOPKINS, M., KAY, C., MCDONALD, A. T., STAPLETON, C. M., WATKINS, J., WILKINSON, J. & WYER, M. D. 2008. Quantification and control of microbial pollution from agriculture: a new policy challenge? *Environmental Science & Policy,* 11**,** 171-184.

KNOWLES, R. 1982. Denitrification. *Microbiological Reviews,* 46**,** 43-70.

KRUPA, S. 2003. Effects of atmospheric ammonia (NH_3) on terrestrial vegetation: a review. *Environmental Pollution,* 124**,** 179-221.

LAMMERTS, E. & GROOTJANS, A. 1997. Nutrient deficiency in dune slack pioneer vegetation: a review. *Journal of Coastal Conservation,* 3**,** 87-94.

MARTINEZ, M. L., PEREZ-MAQUEO, O. & VASQUEZ, V. M. 2004. Facilitative interactions on coastal dunes in response to seasonal weather fluctuations and benefactor size. *Ecoscience,* 11**,** 390-398.

MCLATCHEY, G. P. & REDDY, K. 1998. Regulation of organic matter decomposition and nutrient release in a wetland soil. *Journal of Environmental Quality,* 27**,** 1268-1274.

MERRILL, A. G. & ZAK, D. R. 1992. Factors controlling denitrification rates in upland and swamp forests. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere,* 22**,** 1597-1604.

MYROLD, D. D. 1998. Transformations of nitrogen. *Principles and applications of soil microbiology,* 14**,** 333-373.

OLD, G. H., NADEN, P. S., GRANGER, S. J., BILOTTA, G. S., BRAZIER, R. E., MACLEOD, C. J. A., KRUEGER, T., BOL, R., HAWKINS, J. M. B., HAYGARTH, P. & FREER, J. 2012. A novel application of natural fluorescence to understand the sources and transport pathways of pollutants from livestock farming in small headwater catchments. *Science of the Total Environment,* 417**,** 169-182.

OLFF, H., HUISMAN, J. & VAN TOOREN, B. 1993. Species dynamics and nutrient accumulation during early primary succession in coastal sand dunes. *Journal of Ecology***,** 693-706.

PEACOCK, A. G., MULLEN, M., RINGELBERG, D., TYLER, D., HEDRICK, D., GALE, P. & WHITE, D. 2001. Soil microbial community responses to dairy manure or ammonium nitrate applications. *Soil Biology and Biochemistry,* 33**,** 1011-1019.

PLASSMANN, K., EDWARDS-JONES, G. & JONES, M. L. M. 2009. The effects of low levels of nitrogen deposition and grazing on dune grassland. *Science of the Total Environment,* 407**,** 1391-1404.

PLASSMANN, K., JONES, M. L. M. & EDWARDS-JONES, G. 2010. Effects of long-term grazing management on sand dune vegetation of high conservation interest. *Applied Vegetation Science,* 13**,** 100-112.

PROVOOST, S., JONES, M. L. M. & EDMONDSON, S. E. 2011. Changes in landscape and vegetation of coastal dunes in northwest Europe: a review. *Journal of Coastal Conservation,* 15**,** 207-226.

REMKE, E., BROUWER, E., KOOIJMAN, A., BLINDOW, I., ESSELINK, H. & ROELOFS, J. G. 2009. Even low to medium nitrogen deposition impacts vegetation of dry, coastal dunes around the Baltic Sea. *Environmental Pollution,* 157**,** 792-800.

RHIND, P. & JONES, P. 1999. The floristics and conservation status of sand-dune communities in Wales. *Journal of Coastal Conservation,* 5**,** 31-42.

RHIND, P. & JONES, R. 2009. A framework for the management of sand dune systems in Wales. *Journal of Coastal Conservation,* 13**,** 15-23.

RODWELL, J., DRING, J., AVERIS, A., PROCTOR, M., MALLOCH, A., SCHAMINÉE, J. & DARGIE, T. 2000. Review of coverage of the National Vegetation Classification. *Report-Joint Nature Conservation Committee*.

SIVAL, F., MÜCHER, H. & VAN DELFT, S. 1998. Management of basiphilous dune slack communities in relation to carbonate accumulation and hydrological conditions. *Journal of Coastal Conservation,* 4**,** 91-100.

SMEETS, P., MEDEMA, G. & VAN DIJK, J. 2009. The Dutch secret: how to provide safe drinking water without chlorine in the Netherlands. *Drinking Water Engineering and Science,* 2**,** 1-14.

STEDMON, C. A., MARKAGER, S. & BRO, R. 2003. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. *Marine Chemistry,* 82**,** 239-254.

STRATFORD, C., RATCLIFFE, J., HUGHES, A. G., ROBERTS, J. & ROBINS, N. S. Complex interaction between shallow groundwater and changing woodland, surface water, grazing and other influences in partly wooded duneland in Anglesey, Wales. Proceedings of the CDXXXV congress international association of hydrogeologists: groundwater and ecosystems. *International Association of Hydrogeologists*, 2007. 1-10.

TAHVANAINEN, T. & HARAGUCHI, A. 2013. Effect of pH on phenol oxidase activity on decaying Sphagnum mosses. *European Journal of Soil Biology,* 54**,** 41-47.

UKTAG 2014. United Kingdom Technical Advisory Group on the Water Framework Directive. Technical report on groundwater dependent terrestrial ecosystem (GWDTE) threshold values. V9; 23 June 2014.

UNC, A. & GOSS, M. J. 2003. Movement of faecal bacteria through the vadose zone. *Water Air and Soil Pollution,* 149**,** 327-337.

VADAS, P. A., HARMEL, R. D. & KLEINMAN, P. J. A. 2007. Transformations of soil and manure phosphorus after surface application of manure to field plots. *Nutrient Cycling in Agroecosystems,* 77**,** 83-99.

VAN DEN BERG, L. J., TOMASSEN, H. B., ROELOFS, J. G. & BOBBINK, R. 2005. Effects of nitrogen enrichment on coastal dune grassland: a mesocosm study. *Environmental Pollution,* 138**,** 77-85.

VAN DER LAAN, D. 1979. Spatial and temporal variation in the vegetation of dune slacks in relation to the ground water regime. *Vegetation,* 39**,** 43-51.

VEER, M. 1997. Nitrogen availability in relation to vegetation changes resulting from grass encroachment in Dutch dry dunes. *Journal of Coastal Conservation,* 3**,** 41-48.

WEIER, K., DORAN, J., POWER, J. & WALTERS, D. 1993. Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. *Soil Science Society of America Journal,* 57**,** 66-72.

WETZEL, R. G. 1992. Gradient-dominated ecosystems: sources and regulatory functions of dissolved organic matter in freshwater ecosystems. *Dissolved organic matter in lacustrine ecosystems.* Springer.

WHO 2006. *Guidelines for drinking-water quality: First addendum to volume 1, Recommendations*, World Health Organization.

WILLIS, A. 1963. Braunton Burrows: the effects on the vegetation of the addition of mineral nutrients to the dune soils. *The Journal of Ecology***,** 353-374.

WILLIS, A., FOLKES, B., HOPE-SIMPSON, J. & YEMM, E. 1959. Braunton Burrows: the dune system and its vegetation. *The Journal of Ecology***,** 249-288.

WITHERS, P. J. A., JARVIE, H. P., HODGKINSON, R. A., PALMER-FELGATE, E. J., BATES, A., NEAL, M., HOWELLS, R., WITHERS, C. M. & WICKHAM, H. D. 2009. Characterization of Phosphorus Sources in Rural Watersheds. *Journal of Environmental Quality,* 38**,** 1998-2011.

CHAPTER 2: **Evidence for sensitivity of dune wetlands to groundwater nutrients**

J. Rhymes [1], H. Wallace [2], N. Fenner [1] L. Jones [3]

[1]Bangor University, UK; [2] Ecological Surveys (Bangor), UK; [3]Centre for Ecology and Hydrology, UK

Author Contributions: The chapter was written by Jennifer Rhymes. Laurence Jones, Nathalie Fenner and Hilary Wallace provided advice on the experimental design and gave editorial suggestions.

2.1 Abstract

Dune slacks are seasonal wetlands, high in biodiversity, which experience considerable within-year and between-year variations in water table. They are subject to many pressures including climate change, land use change and eutrophication. Despite their biological importance and the threats facing them, the hydrological and nutrient parameters that influence their soil properties and biodiversity are poorly understood and there have been no empirical studies to date testing for biological effects in dune systems resulting from groundwater nutrients at low concentrations. In this study we examined the impact of groundwater nutrients on water chemistry, soil chemistry and vegetation composition of dune slacks at three distance classes (0-150 m, 150–300 m, 300–450 m) away from known (off-site) nutrient sources at Aberffraw dunes in North Wales, whilst accounting for differences in water table regime. Groundwater nitrate and dissolved inorganic nitrogen (DIN) and soil nitrate all had significantly higher concentrations closest to the nutrient source. Multivariate analysis showed that although plant species composition within this site was primarily controlled by water table depth and water table fluctuation, nitrogen from groundwater also influenced species composition, independently of water table and soil development. A model containing all hydrological parameters explained 17% of the total species variance; an additional 7% was explained following the addition of NO_3 to this model. Areas exposed to elevated, but still relatively low, groundwater nutrient concentrations (mean 0.204 mg l^{-1} ± 0.091 of DIN) had greater abundance of nitrophilous species and fewer basipholous species than in areas with lower concentrations. This shows that clear biological impact occurs below previously suggested DIN thresholds of 0.20 – 0.40 (mg l^{-1}).

2.2 Introduction

Sand dune systems have a global distribution (Martinez et al. 2004) and support a high biodiversity, including many threatened plant, insects and other animal species (Rhind and Jones, 2009; Howe et al. 2010). They contain seasonal wetlands, known as dune slacks, which support a particularly diverse flora in Europe (Grootjans, 2004), including red list species such as the fen orchid *Liparis loeselii* and the liverwort *Petalophyllum ralfsii*.

Sand dune systems have undergone considerable change globally in the last Century (Martinez et al. 2004). In temperate European dune systems these drivers include: changes in land use, crashing rabbit populations, climate change and eutrophication (Provoost et al., 2011; Jones et al. 2011; Beaumont et al. 2014). With regard to the latter; nutrients from atmospheric deposition have increased dramatically from their pre-industrial levels of 2 – 6 kg N ha^{-1} yr^{-1} (Fowler, 2004). As a consequence, the critical load defined for dune slacks, 10-15 kg N ha^{-1} yr^{-1} (Bobbink and Hettelingh, 2010), is exceeded across much of Europe. While the effects of atmospheric deposition have received recent attention in dry dune habitats (Plassmann et al., 2009; Remke et al. 2009; Jones et al. 2013), relatively little attention has been given to the impact of other sources of nutrients in dune wetlands, indeed in wetlands in general, and the issue of groundwater or surface water-derived nutrients is not explicitly considered within atmospheric critical loads frameworks. In dune systems that are not isolated hydrologically from surrounding groundwater, there is the potential for nutrient inputs to these habitats from agricultural and other sources via groundwater to add to the nutrient load already received from atmospheric deposition. A collation of dune groundwater chemistry data (Davy et al., 2010) suggested that values > 1 mg l^{-1} dissolved inorganic nitrogen (DIN) in dune groundwater indicated probable nutrient contamination of the groundwater within a site, while concentrations above 0.2 mg l^{-1} may also signify contamination. A global assessment of aquatic ecosystems concluded that concentrations above 0.5 – 1.0 mg l^{-1} of total nitrogen could lead to eutrophication (Camargo and Alonso, 2006). There have been studies in the Netherlands on impacts of highly eutrophic river water around drinking water infiltration ponds (Meltzer and van Dijk, 1986). However, there have been no empirical studies to date testing for biological effects in dune systems resulting from groundwater nutrients at low concentrations.

Species distribution within these ecosystems is governed primarily by water table depth, seasonal water table fluctuations and water chemistry (Curreli et al., 2012; Grootjans et al., 1996; Lammerts et al., 2001; Lammerts et al., 1992; Willis et al., 1959). Yet, there remains a major knowledge gap as to how groundwater nutrients may affect dune slack vegetation and at what concentrations (Jones

et al. 2006). Studies of atmospheric nitrogen deposition impacts have been made in many habitats (e.g. Phoenix et al. 2012), with the potential for community shift in extreme cases such as conversion of heathlands into grasslands (Heil and Diemont, 1983). However, in dune slacks there is still relatively little empirical evidence of nutrient impacts either from atmospheric deposition or from other sources, especially at realistic N loads. One of the few studies, using high nutrient loads on dune vegetation at Braunton Burrows demonstrated that *Agrostis stolonifera* dominated a dune slack following surface additions of N and P (Willis, 1963).

Dune slack water tables tend to be at their highest in winter and fall in the summer months (Van Der Laan, 1979) as the water table is highly dependent on precipitation and evaporation. Water tables can also vary substantially from year to year (Ranwell, 1959; Stratford et al. 2013), causing periods of drought and flooding which affect the period in which the rooting zone is in contact with the water table. These fluctuations also play an important role in controlling nutrient composition within the soils. During periods of high water level, mineralisation of organic matter is reduced thus conserving the low nutrient status favoured by dune slack species (Berendse et al., 1998). Soil processes are important in regulating the impacts of N. Soil exchange sites may actively bind ammonium from the groundwater during periods of inundation, while denitrifying bacteria may release nitrogen back into the atmosphere (Myrold, 1998).

The aim of this investigation was to examine the impact of nutrients on dune groundwater chemistry, soil chemistry and botanical composition along gradients of nutrient input from known sources, and accounting for differences in water table regime. We tested the following hypotheses: Does nutrient contamination from off-site sources extend into the groundwater under the dune system? If nutrients are present in the groundwater, is there any evidence in the plant assemblages and soils of dune slacks that these nutrients are accessible to the vegetation in the dune slacks, and do they have an adverse ecological impact on the plant community composition?

2.3 Methods

2.3.1 Site Description

Aberffraw dunes are located on the south west corner of the island of Anglesey in North Wales, UK (53°11'N, 4°27'W). The site extends for 1 km in width and 3 km inland (Fig 2.1). A small lake, Llyn Coron bounds the north east edge of the system and feeds the river Afon Fraw, which flows along the north-west edge of the dunes down to the sea. The site is in a low valley surrounded on all sides by agricultural land. The agricultural land is reseeded and fertilised pasture, used for sheep and

cattle grazing, with feed stations on land immediately adjacent to the south-east dune site boundary. A number of streams and ditches draining this heavily fertilised agricultural area lead on to the site.

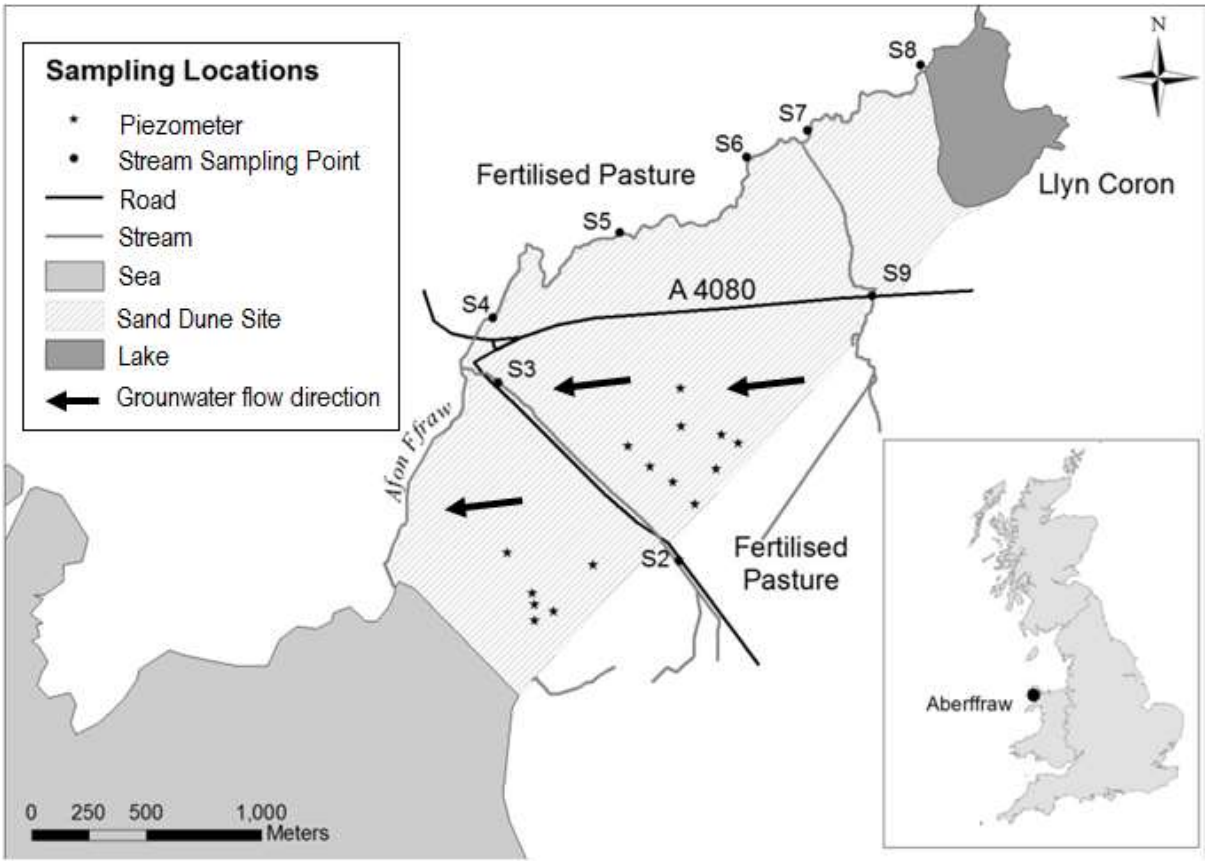


Fig 2.1 Map of Aberffraw dune system, showing all piezometers and stream (S2 - S9) sampling points. S1 (not shown) was an episodic stream and data were only collected from this sampling point for one month. Cross-hatched area represents designated site. Redrawn from Ordnance Survey.

2.3.2 Groundwater flow direction

In a preliminary survey, elevation of the water table at each piezometer and at additional locations around the site was measured by auguring down to the water table and then referred to ground surface elevation measured using a Leica 1200 RTKGPS, with a vertical accuracy of ± 1 cm, and correcting for water table depth. Groundwater flow direction was estimated by contour analysis in ArcGIS v10.1.

2.3.3 Sampling design

A preliminary survey was carried out whereby water samples were collected by drilling down to the water table with an augur and sampling the groundwater with a hand pump. This established that

there was a possible nitrate contamination gradient that extended into the site from the fertilised pastureland on the south-east site boundary. In order to quantify the possible effects of this contamination 15 piezometers, 2 m in depth with full-length slotted screens of 0.3 mm slots covered by mesh were installed. Installation was restricted to dune slack areas as this is where vegetation and rooting zone are in contact with the groundwater and where impacts are most likely to occur. The sampling strategy aimed at evaluating gradients in water chemistry within three distance classes from the south-east site boundary (0 – 150 m, 150 – 300 m and 300 – 450 m).

2.3.4 Hydrological monitoring and water chemistry sampling

Monthly manual measurements of groundwater levels were taken from 15 piezometers using a water level meter (Boart longyear), starting in March 2012 for a period of 12 months. Water samples were collected monthly from the top 10 cm of the water table at each piezometer. During periods of inundation, when water table was above ground level in certain slacks, samples of the standing water above the piezometer were collected. Water samples were also collected from streams entering or nearby the site (Fig 2.1), which could potentially contribute to groundwater nutrients via seepage from the stream bed. Stream water samples were collected at the same time as groundwater, by dipping a clean collecting container into the surface flow. Samples were stored in darkness at 5°C prior to chemical analysis. pH was recorded for each sample which was then filtered through 0.45 µm nylon syringe filter (Avonchem™). Dissolved inorganic anions (chloride, nitrite, nitrate, phosphate and sulphate) and cations (sodium, ammonium, potassium, calcium and magnesium) were then measured on an ion chromatograph (Metrohm, UK Ltd.). Detection limits for all anions and cations were 0.005 mg l^{-1} apart from nitrite (0.003 mg l^{-1}), nitrate (0.002 mg l^{-1}) and ammonium (0.001 mg l^{-1}). Dissolved inorganic nitrogen was calculated as the sum of NO_3-N, NO_2-N and NH_4-N.

2.3.5 Botanical Survey

At each of the 15 piezometers vegetation was surveyed in three 1 m x 1 m quadrats. The quadrats were placed at a 3 m distance from the piezometer and arranged on cardinal bearings (North, West and East). Species occurrence was recorded using visual estimates of % cover for all species of vascular plants, lichens and bryophytes. Nomenclature follows Stace (2010) for vascular plants and Hill et al. (1994) for bryophytes. Cover of bare ground and litter were also recorded. The location of each quadrat was recorded at its centre using a Leica 1200 RTKGPS. Mean UK-modified Ellenberg indicator values (Hill et al., 1999, Hill et al., 2007) were then calculated for each quadrat using species presence data.

2.3.6 Topographical resolution

Elevation of the ground surface at each piezometer and quadrat was measured using the Leica 1200 RTKGPS to 1 cm vertical resolution, which allowed groundwater levels for each quadrat to be calculated using their relative elevation difference from the nearest piezometer.

2.3.7 Soil Sampling

At each quadrat a soil core (5 cm diameter, 15 cm depth) was collected and stored in darkness at 5°C, prior to analysis. The thickness of the organic horizon was recorded and any vegetation and large roots were removed. The soil was then homogenised by hand and a sub-sample (10-15 g field moist soil) was weighed and dried at 105°C and reweighed to measure moisture content. The samples were then re-heated in a furnace at 375°C for 16 h and re-weighed to determine organic matter content through Loss on Ignition (Ball et al. 1964).

A sub-sample was prepared for chemical analysis using a water extraction of 10 g homogenised sample of fresh soil, mixed with 10 ml of ultra-high purity water (1:10 wt/vol) on a laboratory blender (Stomacher 80, Seward UK). pH was recorded using a calibrated pH electrode and electrical conductivity was measured using a conductivity meter (Primo 5, Hanna Instruments Ltd UK).The remaining solution was centrifuged for 15 mins at 5000 rpm and filtered through 0.45 µm nylon syringe filter (Avonchem™). Organic anions (chloride, nitrite, phosphate and sulphate) and cations (sodium, ammonium, potassium, calcium and magnesium) were then measured on the Metrohm ion chromatograph, detection limits described above.

2.3.8 Rooting depth

Soil pits > 30 cm wide and 1 m deep were dug at 5 m distance from six of the piezometers in order to measure rooting depth. Three of these were dug in slacks with a hydrological regime supporting wet slack vegetation communities and three in dry slack communities. On one clean vertical face in each soil pit, the number of visible roots in a 30 cm wide x 20 cm deep section were recorded at 4 depth bands below the surface (-20 to -40 cm, -40 to -60 cm, -60 to -80 cm and -80 to -100 cm). It was not possible to count visible roots in the main rooting zone (top layer 0 to -20 cm) due to the high abundance of roots.

2.3.9 Statistical Analysis

Piezometers were grouped into three classes based on their distance from the south-east site boundary (See Fig 2.1) (0-150 m N= 5, 150-300 m N= 6, 300-450 m N= 4), as were the quadrats

associated with them. Monthly groundwater (including inundation samples) and streamwater (N = 8) chemistry values and pH for each sampling point were averaged to give an annual mean, as preliminary analysis showed no seasonal differences in groundwater chemistry. Data from the soil samples taken from each of the three quadrats around a piezometer were averaged to give a single value associated with each piezometer and were tested for statistical differences among distance classes using ANCOVA (Minitab v16), where the average annual maximum water table elevation from the three quadrats was used as a covariate. Data that proved not normally distributed (Kolmogorov-Smirnov Test) were transformed using a Johnson's transformation (Johnson, 1995). Differences in root abundance between wet and dry slack community types were tested at each of the four rooting depth zones (-20 to -40 cm, -40 to -60 cm, -60 to -80 cm and -80 to -100 cm) using analysis of variance in Minitab v16.

Relationships between vegetation and measured soil and water variables were sought using multivariate analyses. An initial DCA of the 45 vegetation quadrats tested the length and strength of the first gradient (Lengths of gradient axis 1= 3.07, axis 2= 2.36, axis 3= 1.81 and axis 4= 1.56), whilst relationships between vegetation and environmental variables were explored through indirect gradient analysis using PCA. The significance of the relationships with environmental variables was tested singly and within models using Redundancy Analysis (RDA) Monte Carlo methods within CANOCO v 4.5. Mean botanical, soil and hydrological regime data for the three quadrats associated with each piezometer were utilised within the RDA analysis, giving N=15 datapoints.

2.4 Results

2.4.1 Groundwater direction, groundwater and stream chemistry

The preliminary topographical and water level survey showed that the direction of groundwater flow is approximately westerly (Fig 2.1). The summary data of annual piezometer water chemistry (Table 2.1) showed significant differences in annual mean groundwater nitrate concentrations (F= 4.52 df= 2 p= 0.034) of the piezometers in the three classes with those in the 0-150 m class (0.885 ± 0.283 mg l^{-1}) being significantly greater than those in the 150-300 m (0.360 ± 0.147 mg l^{-1}) or 300-450 m classes (0.092 ± 0.046 mg l^{-1}). Significant difference was also found in annual mean groundwater dissolved inorganic nitrogen concentrations (F= 4.54 df= 2 p= 0.034), with those in the 0-150 m class (0.204 ± 0.091 mg l^{-1}) again being significantly greater than those in the 150-300 m (0.084 ± 0.034 mg l^{-1}) or the 300-450 m classes (0.0224 ± 0.011 mg l^{-1}). All other piezometer water chemistry variables showed no significant difference among classes. Nitrate and phosphate concentrations were an

order of magnitude higher in the streams running through the site than in the dune groundwater, even in the class of piezometers nearest the south-east site boundary.

Table 2.1 Summary of annual mean water chemistry from piezometers and streams; values for each variable are expressed as mean ± standard error and brackets show minimum and maximum values. Values in bold show significant differences in groundwater chemistry among distance classes (see text).

Chemistry and pH	**Groundwater**	**Streams**
Chloride ($mg\ l^{-1}$)	68.717 ± 2.249	44.141 ± 2.421
	(16.113, 190.945)	(22.707, 211.436)
Nitrite ($mg\ l^{-1}$)	0.008 ± 0.001	0.042 ± 0.004
	(0.003, 0.185)	(0.005, 0.211)
Nitrate ($mg\ l^{-1}$)	**0.468 ± 0.112**	10.945 ± 1.438
	(0.002, 16.706)	(0.003, 86.833)
Phosphate ($mg\ l^{-1}$)	0.006 ± 0.000	0.058 ± 0.010
	(0.005, 0.041)	(0.005, 0.520)
Sulphate ($mg\ l^{-1}$)	16.887 ± 0.872	14.376 ± 0.699
	(1.088, 77.230)	(1.550, 41.391)
Sodium ($mg\ l^{-1}$)	35.072 ± 1.093	25.806 ± 0.997
	(13.130, 92.294)	(0.005, 123.956)
Ammonium ($mg\ l^{-1}$)	0.036 ± 0.005	0.031 ± 0.005
	(0.001, 0.585)	(0.003, 0.221)
Potassium ($mg\ l^{-1}$)	1.857 ± 0.079	4.398 ± 0.330
	(0.005, 6.223)	(0.005, 15.310)

Chemistry and pH	**Groundwater**	**Streams**
Calcium ($mg\ l^{-1}$)	83.723 ± 1.336	48.486 ± 1.657
	(40.847, 187.050)	(0.020, 94.846)
Magnesium ($mg\ l^{-1}$)	7.086 ± 0.167	8.655 ± 0.184
	(0.005, 14.720)	(0.005, 21.234)
Dissolved inorganic N ($mg\ l^{-1}$)	**0.108 ± 0.002**	2.485 ± 0.030
	(0.001, 3.829)	(0.002, 19.609)
pH	7.511 ± 0.021	7.715 ± 0.027
	(6.804, 8.473)	(7.194, 8.620)

Nitrate concentrations at all stream sampling points (Fig 2.2) do not exceed 20 mg l^{-1} apart from S2 which considerably exceeds this concentration. They show a slight seasonal trend with concentrations lower in summer than in winter. By contrast, the stream S2 which drains from the south-east boundary into the site shows a steep and rapid increase in nitrate concentration from mid-April that exceeds 50 mg l^{-1} for four months, peaking at 87 mg l^{-1} in June before rapidly declining from June to September to concentrations similar to other stream sampling points.

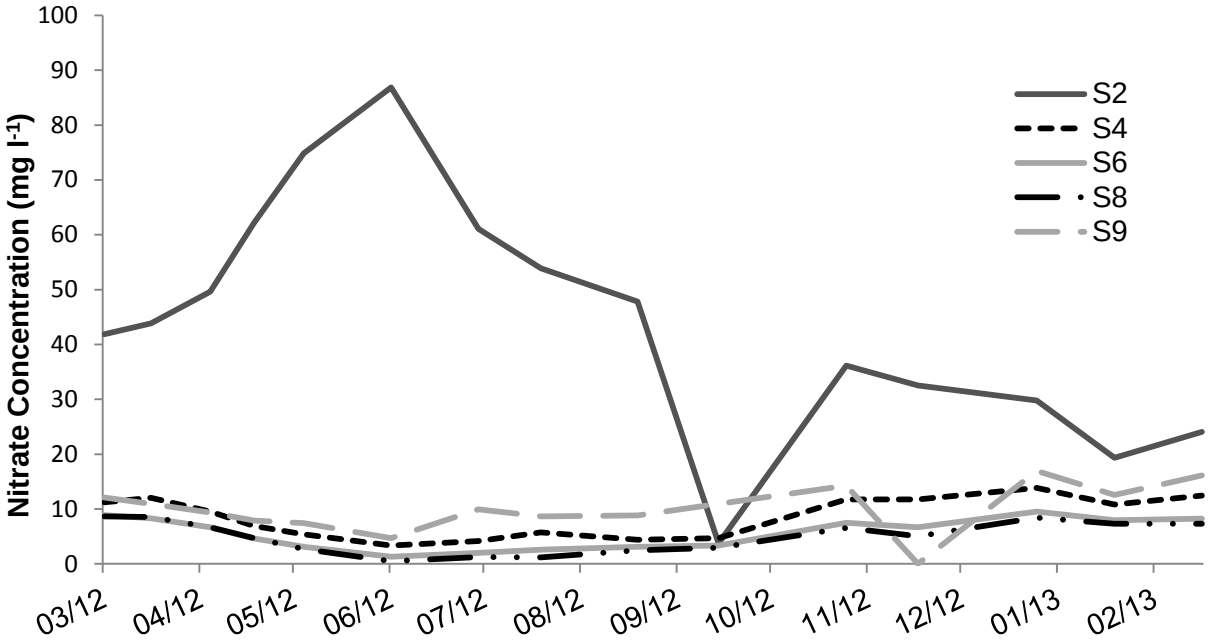


Fig 2.2 Monthly nitrate concentrations (mg l^{-1}) from four representative stream sampling points and from divergent S2 (see Fig 2.1) over a 12 month period. Samples from streams S3, S5, S7 not shown for clarity, but were not significantly different from S4-S9 shown here.

2.4.2 Soils

A summary of soil physico-chemistry parameters for the three classes of quadrats are shown in Table 2.2. Significantly higher (F= 5.27 df= 2 p= 0.025) soil nitrate concentrations occurred in the 0-150 m class the 0-150m class (1.967± 0.515 µg g ¯1 dry soil) compared with that of both the 150-300 m (0.612 ± 0.155 µg g ¯1 dry soil) and 300-450 m class (1.087± 0.730 µg g ¯1 dry soil). No significance among classes was found for soil dissolved inorganic nitrogen, nitrite, ammonium and for all other variables.

Table 2.2 Summary of soil physico-chemistry parameters for categorised quadrats located 0-150 m (N = 5), 150-300 m (N = 6) and 300-450 m from within the south-east site boundary (N = 4). Values for each variable are expressed as mean ± standard error; brackets show minimum and maximum values for each class. Significant differences among distance classes shown in bold; values denoted by the same letter not significantly different from each other.

Variable		Distance from Fence		
		0-150	**150-350**	**350-500**
		n=5	**n=6**	**n=4**
Soil Chemistry (µg g^{-1} dry soil)	Chloride	1.653 ± 0.048 (1.539, 1.803)	1.473 ± 0151 (1.033, 2.149)	1.794 ± 0.307 (1.299, 2.686)
	Nitrite	0.036 ± 0.016 (0.011, 0.097)	0.013 ± 0.005 (0.004, 0.037)	0.019 ± 0.014 (0.003, 0.060)
	Nitrate	**0.784 ± 0.264^{A} (0.190, 1.725)**	**0.237 ± 0.089^{AB} (0.081, 0.640)**	**0.166 ± 0.081^{B} (0.046, 0.406)**
	Phosphate	0.007 ± 0.002 (0.005, 0.015)	0.005 ± 0.000 (0.005, 0.007)	0.005 ± 0.000 (0.005, 0.005)
	Sulfate	1.454 ± 0.117 (1.113, 1.808)	1.463 ± 0.089 (1.149, 1.801)	1.500 ± 0.163 (1.062, 1.780)
	Sodium	1.123 ± 0.287 (0.005, 1.617)	1.330 ± 0.432 (0.196, 2.660)	1.266 ± 0.357 (0.737, 2.270)
	Ammonium	0.141 ± 0.028 (0.090, 0.248)	0.145 ± 0.034 (0.063, 0.290)	0.254 ± 0.115 (0.066, 0.583)
	Potassium	1.559 ± 0.341 (0.620, 2.510)	1.740 ± 0.212 (1.201, 2.519)	2.496 ± 1.003 (1.009, 5.423)
	Calcium	11.736 ± 2.386 (5.530, 17.770)	11.286 ± 1.843 (3.451, 15.696)	11.577 ± 1.543 (7.140, 14.043)
	Magnesium	0.691 ± 0.132 (0.380, 1.104)	0.856 ± 0.097 (0.519, 0.655)	0.868 ± 0.132 (0.655, 1.209)
	Total Inorganic N	0.332 ± 0.127 (0.131, 0.822)	0.183 ± 0.044 (0.069, 0.373)	0.242 ± 0.106 (0.063, 0.527)
Soil Characteristics	pH	7.690 ± 0.248 (6.962, 8.146)	7.990 ± 0.180 (7.153, 8.436)	8.054 ± 0.144 (7.660, 8.301)
	Electrical conductivity	35.067 ± 4.303 (19.333, 44.000)	25.111 ± 4.977 (14.667, 45.667)	33.250 ± 0.886 (13.000, 34.667)
	LOI %	3.791 ± 0.355 (2.806, 4.955)	5.947 ± 0.529 (3.827, 7.270)	4.354 ± 0.682 (2.902, 6.065)
	Observed organic matter	8.933 ± 1.416 (5.000, 12.667)	10.890 ± 1.108 (8.667, 15.000)	7.500 ± 1.303 (5.500, 11.333)

2.4.3 Vegetation

Figure 2.3 shows water table variation in relation to rooting depth for two hydrographs typical of a wet and a dry slack community, corresponding to UK National Vegetation Classification SD14d (*Salix repens-Campyllium stellatum* dune slack, *Festuca rubra* sub-community) and SD15b (*Salix repens-Calliergon cuspidatum* dune slack, *Equisetum variegatum* sub-community) respectively (Rodwell 2000). Both wet and dry slack communities reveal an asymmetric seasonal pattern whereby the water depth drops steadily over the summer period and increases rapidly in early winter (Fig 2.3). The dry slack community has a lower water depth than that of a wet slack all year around, with the greatest differentiation occurring in the drier months of summer (Fig 2.3). Roots were significantly more abundant at depths -40 to -60 cm in dry communities than in wet communities (Table 2.3), there was no significant difference at all other depths. In the wet slack vegetation community, the main rooting zone (0 to -40 cm) is in contact with the water table for approximately 8 months (Fig 2.3. In the dry slack vegetation community, the main rooting zone (0 to –60 cm) is also in contact with the water table for a similar duration (Fig 2.3), suggesting rooting depth is constrained by water levels.

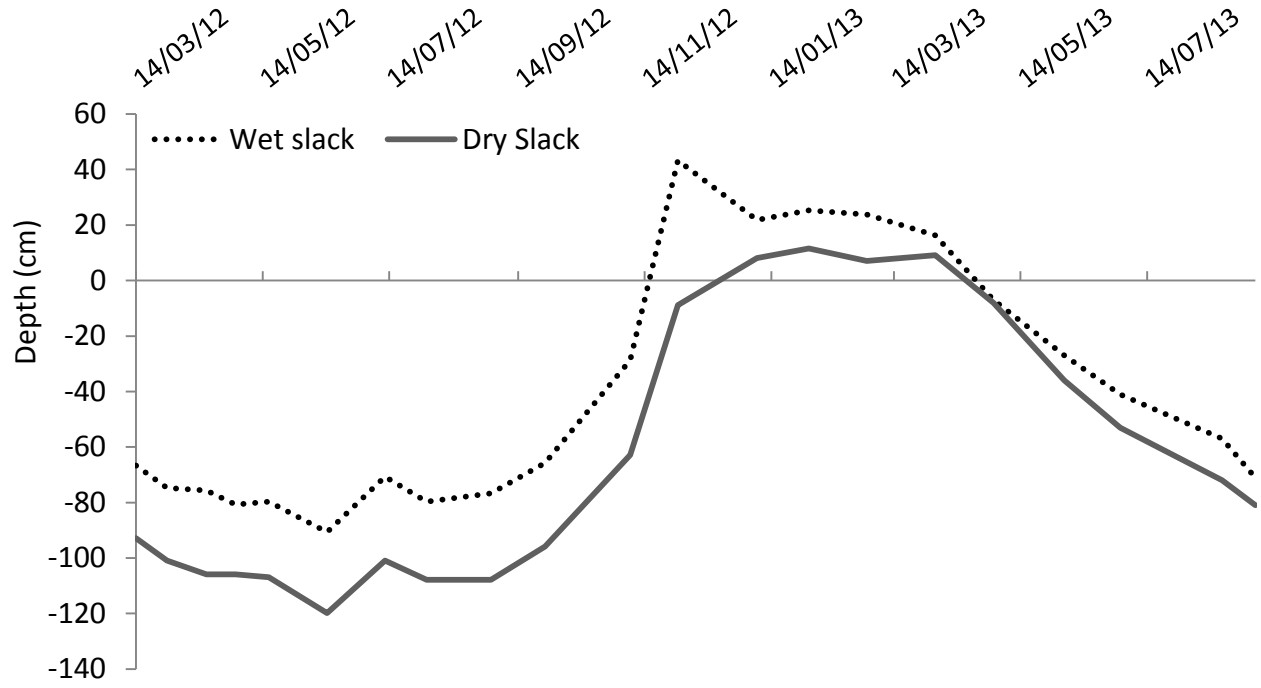


Fig 2.3 Annual hydrographs of two piezometers from a wet SD 15b and a dry SD 14d slack.

Table 2.3 Visible roots observed at 4 rooting depth zones (-20 to -40 cm, -40 to -60 cm, -60 to -80 cm and -80 to -100 cm) expressed as mean ± SE. Letters denote significant difference between root abundance in dry slacks SD 14d and wet slacks SD 15b.

Rooting depth zone (cm)	Observed roots	
	Wet slack	Dry Slack
-20 to -40	16.33 ± 4.29	30.00 ± 4.22
-40 to -60	**4.67 ± 0.96** [A]	**10.67 ± 0.69** [B]
-60 to -80	4.16 ± 0.33	4.33 ± 1.39
-80 to -100	0.33 ± 0.19	0.67 ± 0.19

The PCA plot shows the distribution of the 45 quadrats, coded by their distance to the south-east site boundary, with environmental variables overlain to aid interpretation (Fig 2.4). The overlain environmental variables suggest that axis 1 (Fig 2.4 a) relates to a hydrological gradient in which annual maximum water level, water level range and Ellenberg F were negatively associated with the axis, i.e. high water tables were found to the left of the diagram corresponding to low scores on axis 1. The low axis 1 scores (Fig 2.4 b) were occupied by species tolerant of wet soils *Galium palustre*, *Hydrocotyle vulgaris* and *Carex nigra* whereas the highest axis 1 scores were occupied by species characteristic of drier sites such as *Lotus corniculatus* and *Trifolium repens*. Axis 2 (Fig 2.4 a) related to a combined soil development/nutrient axis where groundwater NO_2 concentrations, soil NO_3 concentrations and Ellenberg N were positively linked with axis 2, and soil pH and Ellenberg R were negatively associated with the axis. The overlain species data reinforce this pattern, with low axis 2 scores (Fig 2.4 b) occupied by species with higher base status demand such as *Campyllium stellatum* and *Equisetum variegatum* whereas high scores were revealed by higher fertility species e.g. *Rubus caesius* and *Potentilla reptans.* There was no clear separation of quadrats relative to distance to south-east site boundary on Axis 1 (Fig 2.4 a), however axis 2 segregated the 0-150 m class from the 300-450 m class, with quadrats in the 0-150 m class located higher on axis 2.

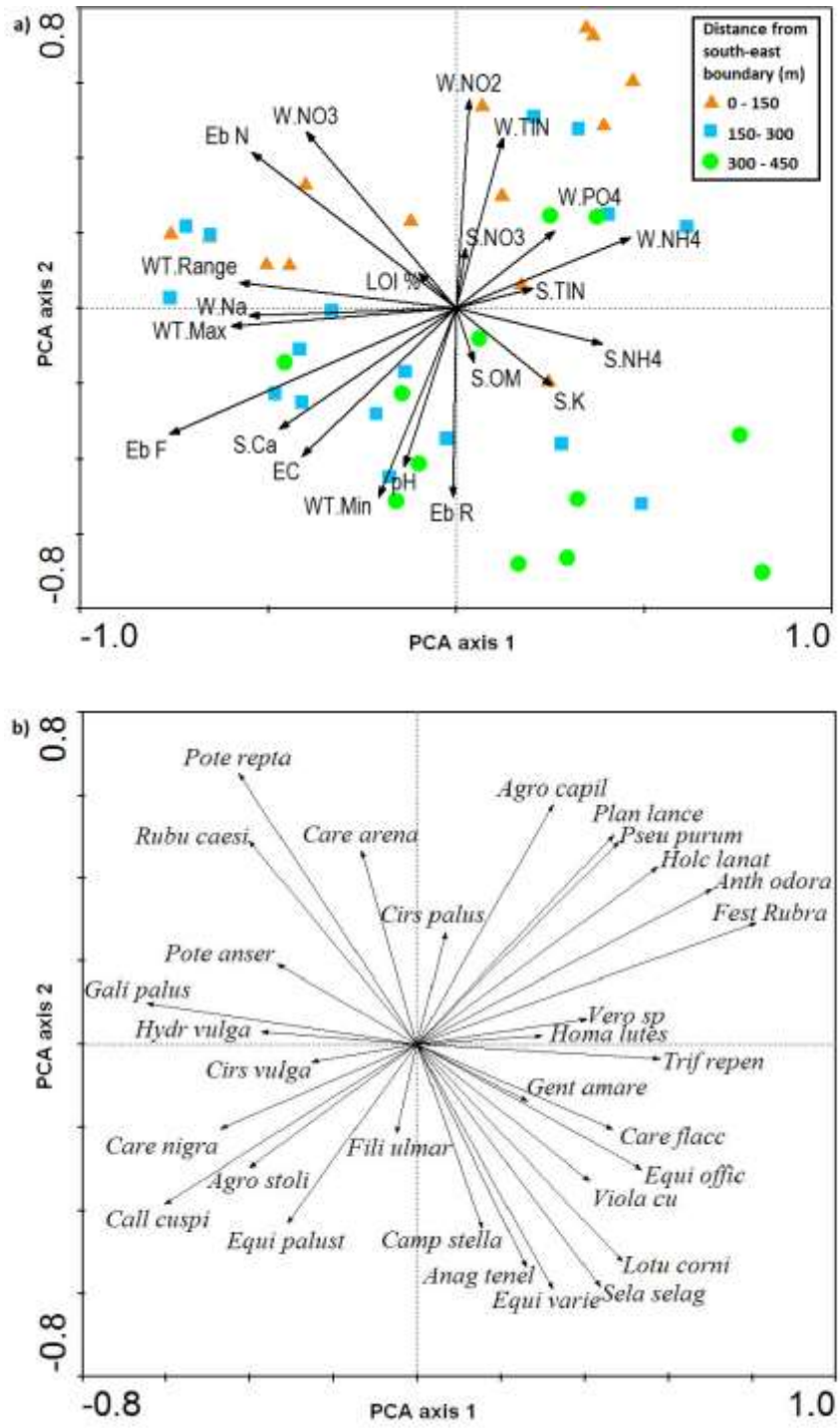


Fig 2.4 PCA analysis: (a) the distribution of environmental variables with PCA scores, quadrats coded by distance to south-east site boundary. Only environmental variables and species with axis scores >0.2 are shown for clarity (except LOI). See Tables 2.2 and 2.3 for full list of variables. Prefixes denote the following S- Soil; W-groundwater and WT- water table and pH -soil pH. The percentage variation explained by axis 1= 20.1%, axis 2= 35.7%, axis 3= 43.5% and axis 4= 51.0%. For full names of species see Table A1 (Appendix IV) in the supplementary material.

Using a Monte Carlo permutation test the model containing all variables was highly significant ($p<0.001$), where the first four axes explained 51.0% of the species-environment relationships and explained 45.9% of the total species variance. Various hydrological variables, soil variables and water chemistry when tested singly were significant (Table 2.4). A model containing all hydrological parameters explained 19.9% of the total species variance, showing as expected a degree of co-correlation between hydrological variables. When tested singly, NO_3 explained more of the total species variance than any of the individual hydrological variables. When the influence of all hydrological variables was accounted for in a combined model, adding NO_3 explained an additional 8.2% of species variance. This shows that species variation due to groundwater NO_3 was largely independent of that due to hydrology, and that NO_3 was significantly affecting plant community composition.

Table 2.4 Environmental variables illustrating percentage of total species variation explained within RDA and significance, when tested singly.

Variables		**Variance (%)**	**significance**
Hydrological variables	Annual maximum water level (m)	11.1%	*
	Annual Range (m)	10.5%	
	Annual minimum water level (m)	9.7%	
Soil variables	EC (mS/cm^{-1})	15.0%	**
	S.NH_4 ($\mu g\ g^{-1}$ dry soil)	13.2%	*
	S.Ca ($\mu g\ g^{-1}$ dry soil)	12.3%	*
	S.pH	11.4%	*
	S.DIN ($\mu g\ g^{-1}$ dry soil)	10.5%	
	S.Mg ($\mu g\ g^{-1}$ dry soil)	10.9%	
	Soil moisture (%)	8.7%	
	LOI (%)	5.7%	

Variables		Variance (%)	significance
Water chemistry	W.PO_4 (mg l^{-1})	14.9%	***
	W.NH_4 (mg l^{-1})	11.3%	*
	W.NO_3 (mg l^{-1})	11.2%	*
	W.Na (mg l^{-1})	11.2%	*
	W.Cl (mg l^{-1})	9.3%	
	W.DIN (mg l^{-1})	8.3%	
	W.NO_2 (mg l^{-1})	7.3%	
	W.K (mg l^{-1})	7.0%	
	W.Br (mg l^{-1})	7.0%	
	W.SO_2 (mg l^{-1})	6.1%	
	W.Ca (mg l^{-1})	5.7%	
	W.Mg (mg l^{-1})	5.4%	
Combined models	Hydrological parameters (min, max + range)	19.9%	*
	Hydrological parameters and groundwater nitrate (min, max, range, W.$NO_{3)}$	28.1%	*

* significant at 0.05 level

** significant at 0.01 level

*** significant at 0.001 level

2.5 Discussion

This study has shown that there is a nutrient contamination gradient that extends from the south-east site boundary into the site which is significantly affecting groundwater nitrate and dissolved inorganic N concentrations, soil nitrate and nitrite concentrations and vegetation composition.

Results suggest that the contamination is sourced from the south-east fertilised pasture land and is likely to be due to fertiliser application. Concentrations of nitrate samples from stream S2, which

flows onto the site, exceed the 50 mg l^{-1} nitrate vulnerable zones designation threshold (Environment Agency, 2012) and the 50 mg l^{-1} World Health Organisation's guideline value for drinking water. Contamination is not likely to be due to manure within the site, or from the adjoining pasture land as ammonium and phosphate concentrations are relatively low within the streams, groundwater and soils. In sandy soils with low water holding capacity it is probable that nitrate is rapidly leached post fertiliser application (Skiba and Wainright, 1984), particularly after heavy periods of rainfall and in turn is contaminating the groundwater. The sandy nature of the pasture land at Aberffraw allows groundwater to carry pollutants in a westerly direction into the site but the flow rate is unknown, although a study carried out at a nearby site, Newborough Warren, determined that groundwater flows at a speed of 39.6 m/year (Betson et al., 2002). This suggests at least 3 years of contamination as NO_3 concentrations are elevated at up to 150 m into the site. Although knowledge of the local land management history suggests that the adjacent farmland has been intensively managed for several decades and such nutrient concentrations are unlikely to be a recent phenomenon. Since nitrate concentrations determined from stream S2 are much greater than those determined in the groundwater, this suggests that the spatial extent of contamination could represent a number of possibilities: 1) an equilibrium caused by physical dilution and mixing with uncontaminated rainwater that infiltrates through the sand or 2) a result of processing and denitrifying N within the sandy body, or 3) A combination of both processes. Further work is required to assess to what extent dilution and denitrification play a significant role in reducing NO_3 concentrations in this aquifer.

The observed nitrate and nitrite soil gradient is likely to be due to uptake from the groundwater during the winter and spring months, when water tables are at their highest and plant roots are in direct contact with the groundwater or capillary fringe. This allows for possible nutrient uptake by the plants and subsequently the nutrients return to the soil surface via litter fall (Berendse et al., 1998), and direct binding of ammonium by the soil. As farming on this pastureland has been carried out for decades it is likely that the nutrient gradient has accumulated over time, but has not yet lead to significantly increased organic matter accumulation. This could be due to microbial processes maintaining low nutrient levels, as denitrification has been found to significantly increase with NO_3 availability (Merrill and Zak, 1992). It is also likely that in areas of contamination a shift in the microbial community composition has occurred, which supports higher levels of microbial activity (Peacock et al., 2001) and therefore maintaining low organic matter build up. Although denitrification rates can also be limited by other nutrients such as available carbon (Weier et al., 1993).

Assessment of the rooting zones has determined that the water table is likely to be a major factor controlling the rooting depth and as a result the main rooting zones within wet slacks are found in the shallower 0 to -40 cm zone compared with those within dry slacks in the deeper -0 to -60 cm zone. With differing water table regimes in both communities and the effects of capillary reaction, which carries substantial amounts of water 45 cm above it (Ranwell, 1959), both wet and dry slacks main rooting zones are exposed to groundwater for similar periods of the year and therefore are equally vulnerable to groundwater contamination.

Although the main determinant of species composition was water table depth and water table fluctuation, in broad agreement with the literature (e.g. Lammerts et al. 2001), RDA analysis showed that N was strongly influencing species composition independently of water table and soil development. The results suggest that with increasing availability of N basiphilous species have decreased, while species with higher nutrient status have increased.

If nitrogen pollution within this system continues it is likely that over time the slacks will become more eutrophic, resulting in greater productivity, more rapid soil development, increase in succession rate and loss of species (Jones et al., 2008). Other issues of concern are the projected changes in hydrological regimes, due to climate change, from wet dune slack regimes to dry grassland regimes (Curreli et al., 2012). This is likely to increase the mineralisation of organic matter, such that the desired low nitrogen and phosphorus conditions are not preserved (Lammerts and Grootjans, 1997), which will further exacerbate the eutrophication issue. This study is the first evidence that shows biological impact caused by DIN groundwater concentrations below 0.2 mg l^{-1} within dune wetlands, which is below threshold concentrations described by Davy et al. (2010) and Camargo and Alonso (2006).

2.6 Implications for management

Sandy soils contain very little organic matter or cation exchange sites and therefore have low potential to store nitrogen in the soil, and leach nitrate readily (Rowell, 1994). As a result, it is more cost effective for farmers operating on sandy soils to only apply enough nitrogen that can be directly utilised by the crop. Site specific measures to reduce excess N leaving the site could include a new fertiliser application regime whereby less fertiliser is applied in more frequent doses which will reduce the loss of nitrate through leaching, and installation of buffer zones along pastureland edges and ditches to enhance filtration of nutrients and decrease the rates of runoff (Patty et al., 1997).

2.7 Conclusions

Aberffraw dune system is exposed to a nutrient gradient in groundwater which is likely to be caused by farming practices on surrounding pastureland. Plant species composition of dune slack wetlands within this site is primarily controlled by water table depth and water table fluctuation. However nitrogen from groundwater is influencing species composition independently of water table and soil development, with evidence of an increase in more eutrophic species and a decrease in basiphilous species in affected areas. While there is increasing evidence of N impacts in dry dune habitats (e.g. Jones et al. 2004; van den Berg et al. 2005; Kooijman 2004; Jones et al. 2013), this is the first field-based evidence for impacts of N in dune slacks at relatively low groundwater nutrient concentrations. This study highlights two key findings: impacts have been observed at very low nutrient concentrations of around 0.2 mg l^{-1} DIN, reinforcing potential impacts on aquatic systems at low levels of N (Camargo and Alonso, 2006). Further, it shows that groundwater nutrient inputs need to be considered in addition to atmospheric N inputs in wetland systems. However, additional work is needed to determine the fluxes of N entering the site, in order to match the critical load approach. Experimental approaches to investigate groundwater nutrient impacts would also be useful, but technically difficult to implement.

2.8 References

BALL, D. F. 1963. The soils and land use of the district around Bangor and Beaumaris [sheets 94 and 106]. The soils and land use of the district around Bangor and Beaumaris [sheets 94 and 106].

BEAUMONT, N., JONES, L., GARBUTT, A., HANSOM, J. & TOBERMAN, M. 2014. The value of carbon sequestration and storage in coastal habitats. *Estuarine, Coastal and Shelf Science*, 137, 32-40.

BERENDSE, F., LAMMERTS, E. & OLFF, H. 1998. Soil organic matter accumulation and its implications for nitrogen mineralization and plant species composition during succession in coastal dune slacks. *Plant Ecology*, 137, 71-78.

BETSON, M., CONNELL, M. & BRISTOW, C. 2002. Groundwater modelling of Newborough Warren: A report for the Countryside Council for Wales, Volume 5, Hydrogeology. *The Impact of Forestry on Coastal Geomorphology at Newborough Warren/Ynys Llanddwyn NNR, SSSI, pSAC*.

BOBBINK, R. & HETTELINGH, J.-P. Review and revision of empirical critical loads and dose-response relationships. *Proceedings of an expert workshop, Noordwijkerhout*, 2010. 23-25.

CAMARGO, J. A. & ALONSO, A. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International*, 32, 831-849.

CURRELI, A., WALLACE, H., FREEMAN, C., HOLLINGHAM, M., STRATFORD, C., JOHNSON, H. & JONES, L. 2013. Eco-hydrological requirements of dune slack vegetation and the implications of climate change. *Science of the Total Environment*, 443, 910-919.

DAVY, A., HISCOCK, K., JONES, M., LOW, R., ROBINS, N. & STRATFORD, C. 2010. Ecohydrological guidelines for wet dune habitats, phase 2. Protecting the plant communities and rare species of dune wetland systems. *Bristol, UK: Environment Agency*.

ENVIRONMENT AGENCY. 2012. Method statement for nitrate vulnerable zone review. Surface waters environment agency report to Defra and Welsh Government — supporting paper for the implementation of the nitrates directive 2013–2016.

FOWLER, D., O'DONOGHUE, M., MULLER, J., SMITH, R., DRAGOSITS, U., SKIBA, U., SUTTON, M. & BRIMBLECOMBE, P. 2005. A chronology of nitrogen deposition in the UK between 1900 and 2000. *Water, Air, & Soil Pollution*: Focus, 4, 9-23.

GROOTJANS, A., ADEMA, E., BEKKER, R. & LAMMERTS, E. 2004. Why coastal dune slacks sustain a high biodiversity. In: Martinez ML, Psuty NP (eds) Coastal dunes: *Ecology and conservation. Ecological studies 171 Springer*, Berlin, pp 85–102

GROOTJANS, A. P., SIVAL, F. P. & STUYFZAND, P. J. 1996. Hydro-geochemical analysis of a degraded dune slack. *Vegetation*, 126, 27-38.

HEIL, G. & DIEMONT, W. 1983. Raised nutrient levels change heathland into grassland. Vegetation, 53, 113-120.

HILL, M. O., MOUNTFORD, J., ROY, D. & BUNCE, R. G. H. 1999. Ellenberg's indicator values for British plants. *ECOFACT Volume 2 Technical Annex, Institute of Terrestrial Ecology*.

HILL, M. O., PRESTON, C., BOSANQUET, S. D. & ROY, D. 2007. BRYOATT: attributes of British and Irish mosses, liverworts and hornworts, Centre for Ecology and Hydrology.

HILL, M. O., PRESTON, C. D. & SMITH, A. J. E. 1994. Atlas of the bryophytes of Britain and Ireland, Harley books.

HOWE, M., KNIGHT, G. & CLEE, C. 2010. The importance of coastal sand dunes for terrestrial invertebrates in Wales and the UK, with particular reference to aculeate Hymenoptera (bees, wasps & ants). *Journal of Coastal Conservation*, 14, 91-102.

JONES, L., ANGUS, S., COOPER, A., DOODY, P., EVERARD, M., GARBUTT, A., GILCHRIST, P., HANSOM, J., NICHOLLS, R. J. & PYE, K. 2011. *Coastal margins. In: UK National ecosystem assessment, UNEP-WCMC, Cambridge*.

JONES, L., NIZAM, M. S., REYNOLDS, B., BAREHAM, S. & OXLEY, E. R. B. 2013. Upwind impacts of ammonia from an intensive poultry unit. *Environmental Pollution*, 180, 221-228.

JOHNSON, D. H. 1995. Statistical sirens: the allure of nonparametrics. *Ecology*, 1998-2000.

JONES, M., REYNOLDS, B., BRITTAIN, S., NORRIS, D., RHIND, P. & JONES, R. 2006. Complex hydrological controls on wet dune slacks: the importance of local variability. *Science of the Total Environment*, 372, 266-277.

JONES, M. L. M., SOWERBY, A., WILLIAMS, D. L. & JONES, R. E. 2008. Factors controlling soil development in sand dunes: evidence from a coastal dune soil chronosequence. *Plant and Soil*, 307, 219-234.

JONES, M. L. M., WALLACE, H. L., NORRIS, D., BRITTAIN, S. A., HARIA, S., JONES, R. E., RHIND, P. M., REYNOLDS, B. R. & EMMETT, B. A. 2004. Changes in vegetation and soil characteristics in coastal sand dunes along a gradient of atmospheric nitrogen deposition. *Plant Biology*, 6, 598-605.

KOOIJMAN, A. 2004. Environmental problems and restoration measures in coastal dunes in the Netherlands. M.L. Martínez,, N.P. Psuty, (Eds.), *Coastal dunes: ecology and conservation, Springer-Verlag* (2004), pp. 243–258.

LAMMERTS, E. & GROOTJANS, A. 1997. Nutrient deficiency in dune slack pioneer vegetation: a review. *Journal of Coastal Conservation*, 3, 87-94.

LAMMERTS, E., MAAS, C. & GROOTJANS, A. 2001. Groundwater variables and vegetation in dune slacks. *Ecological Engineering*, 17, 33-47.

LAMMERTS, E., SIVAL, F., GROOTJANS, A. & ESSELINK, H. 1992. Hydrological conditions and soil buffering processes controlling the occurrence of dune slacks species on the Dutch Wadden Sea islands. *In: Carter, R.W.G., Curtis, T.G.E. & Sheehy-Skeffington, M.J. (Eds.), Coastal Dunes: 265-272. Balkema Rotterdam*.

MARTINEZ, M. L., PSUTY, N. P. & LUBKE, R. A. 2008. A perspective on coastal dunes. In: MARTINEZ, M. L. & PSUTY, N. P. (eds.) *Ecological Studies*.

MELTZER, J. & VAN DIJK, H. 1986. The effects of dissolved macro-nutrients on the herbaceous vegetation around dune pools. *Vegetation*, 65, 53-61.

MERRILL, A. G. & ZAK, D. R. 1992. Factors Controlling Denitrification Rates in Upland and Swamp Forests. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, 22, 1597-1604.

MYROLD, D. D. 1998. Transformations of nitrogen. *Principles and Applications of Soil Microbiology*, 14, 333-373.

PATTY, L., REAL, B. & GRIL, J. J. 1997. The use of grassed buffer strips to remove pesticides, nitrate and soluble phosphorus compounds from runoff water. *Pesticide Science*, 49, 243-251.

PEACOCK, A. G., MULLEN, M., RINGELBERG, D., TYLER, D., HEDRICK, D., GALE, P. & WHITE, D. 2001. Soil microbial community responses to dairy manure or ammonium nitrate applications. *Soil Biology and Biochemistry*, 33, 1011-1019.

PHOENIX, G. K., EMMETT, B. A., BRITTON, A. J., CAPORN, S. J., DISE, N. B., HELLIWELL, R., JONES, L., LEAKE, J. R., LEITH, I. D. & SHEPPARD, L. J. 2012. Impacts of atmospheric nitrogen deposition: responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments. *Global Change Biology*, 18, 1197-1215.

PLASSMANN, K., EDWARDS-JONES, G. & JONES, M. L. M. 2009. The effects of low levels of nitrogen deposition and grazing on dune grassland. *Science of the Total Environment*, 407, 1391-1404.

PROVOOST, S., JONES, M. L. M. & EDMONDSON, S. E. 2011. Changes in landscape and vegetation of coastal dunes in northwest Europe: a review. *Journal of Coastal Conservation*, 15, 207-226.

RANWELL, D. 1959. Newborough Warren, Anglesey: I. The dune system and dune slack habitat. The *Journal of Ecology*, 571-601.

REMKE, E., BROUWER, E., KOOIJMAN, A., BLINDOW, I., ESSELINK, H. & ROELOFS, J. G. 2009. Even low to medium nitrogen deposition impacts vegetation of dry, coastal dunes around the Baltic Sea. *Environmental Pollution*, 157, 792-800.

RHIND, P. & JONES, R. 2009. A framework for the management of sand dune systems in Wales. *Journal of Coastal Conservation*, 13, 15-23.

RODWELL, J., DRING, J., AVERIS, A., PROCTOR, M., MALLOCH, A., SCHAMINÉE, J. & DARGIE, T. 2000. Review of coverage of the National Vegetation Classification. *Report-Joint Nature Conservation Commitee.*

ROWELL, D. L. 1994. Soil science: *Methods & applications, Routledge*.

SKIBA, U. & WAINWRIGHT, M. 1984. Nitrogen transformation in coastal sands and dune soils. *Journal of Arid Environments*, 7, 1-8.

STACE, C. 2010. New flora of the British Isles, Cambridge University Press.

STRATFORD, C. J., ROBINS, N. S., CLARKE, D., JONES, L. & WEAVER, G. 2013. An ecohydrological review of dune slacks on the west coast of England and Wales. *Ecohydrology*, 6, 162-171.

VAN DEN BERG, L. J., TOMASSEN, H. B., ROELOFS, J. G. & BOBBINK, R. 2005. Effects of nitrogen enrichment on coastal dune grassland: a mesocosm study. *Environmental Pollution*, 138, 77-85.

VAN DER LAAN, D. 1979. Spatial and temporal variation in the vegetation of dune slacks in relation to the ground water regime. *Vegetation*, 39, 43-51.

WEIER, K., DORAN, J., POWER, J. & WALTERS, D. 1993. Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. *Soil Science Society of America Journal*, 57, 66-72.

WILLIS, A. 1963. Braunton Burrows: the effects on the vegetation of the addition of mineral nutrients to the dune soils. *The Journal of Ecology*, 353-374.

WILLIS, A., FOLKES, B., HOPE-SIMPSON, J. & YEMM, E. 1959. Braunton Burrows: the dune system and its vegetation. *The Journal of Ecology*, 249-288.

CHAPTER 3: **Using chemical, microbial and fluorescence techniques to understand contaminant sources and pathways to wetlands in a conservation site.**

J. Rhymes *[1], L. Jones [2], D.J. Lapworth [3], D. White [3], N. Fenner [1], J.E. McDonald [1], T.L. Perkins [1]

[1]Bangor University, UK; [2]Centre for Ecology and Hydrology, UK; [3] British Geological Survey, UK

Author contributions: The chapter was written by Jenny Rhymes. Laurence Jones, Nathalie Fenner, James McDonald and Dan Lapworth provided advice on the experimental design and editorial suggestions. Dan Lapworth, Debbie White and Tracy Perkins provided technical laboratory advice.

3.1 Abstract

Nutrients and faecal contaminants can enter wetland systems in a number of ways, with both biological and potentially human-health implications. In this study we used a combination of inorganic chemistry, dissolved organic matter (DOM) fluorescence and *Escherichia coli* and total coliform (TCS) count techniques to study the sources and multiple pathways of contamination affecting a designated sand dune site of international conservation importance, surrounded by agricultural land. Analysis of stream samples, groundwater and dune slack wetlands revealed multiple input pathways. These included riverbank seepage, runoff events and percolation of nutrients from adjacent pasture into the groundwater, as well as some on-site sources. The combined techniques showed that off-site nutrient inputs into the sand dune system were primarily from fertilisers, revealed by high nitrate concentrations, and relatively low tryptophan-like fulvic-like ratios < 0.4 Raman units (R.U.). The *E. coli* and TCS counts recorded across the site confirm a relatively minor source of bacterial and nutrient inputs from on-site grazers. Attenuation of the nutrient concentrations in streams, in groundwater and in run-off inputs occurs within the site, restoring healthier groundwater nutrient concentrations showing that contaminant filtration by the sand dunes provides a valuable ecosystem service. However, previous studies show that this input of nutrients has a clear adverse ecological impact.

3.2 Introduction

The global availability and mobility of nitrogen has increased rapidly over the past five decades (Galloway and Cowling, 2002) and the damaging impacts it has on freshwater ecosystems are widely documented (Camargo and Alonso, 2006). Aquatic systems are extremely sensitive to nitrogen and are threatened by atmospheric deposition inputs (Fowler et al., 2005) as well as point sources and diffuse sources which can enter aquatic systems via numerous pathways such as through runoff, streams and groundwater. Tracing sources of aquatic pollution is therefore often problematic (Withers et al., 2009).

Within rural areas river water quality (Hooda et al., 2000) and groundwater quality (Oakes et al., 1981) are primarily impacted by agricultural diffuse pollution (Novotny, 1999). Atmospheric nutrients have been demonstrated to have adverse impacts on the ecology of protected dune habitats (Jones et al., 2013, Plassmann et al., 2008, Field et al., 2014). However, the specific impacts of relatively low levels of nutrients from groundwater on aquatic habitats in dune systems have only recently been documented (Rhymes et al. 2014). As well as nutrients, diffuse inputs of dissolved organic matter (DOM), and micro-organisms into groundwater and surface waters also occur via runoff, field drainage and leaching, as a result of agricultural practices such as slurry and fertiliser application. Previously, these diffuse inputs have largely been characterised by using nutrients as a proxy (e.g. Vadas et al., 2007), although more recent studies are now examining diffuse sources and pathways by investigating pathogenic micro-organisms (Kay et al., 2008) and characterising DOM by natural fluorescence (Hudson et al., 2007). To date there have been no studies combining chemical, fluorescent and microbial techniques to help decipher multiple diffuse sources and pathways. Excitation emission matrix fluorescence spectroscopy (EEMS) can be used to trace DOM from agricultural sources (Baker, 2002, Old et al., 2012). EEMS is sensitive enough to characterise fulvic-like, humic-like and protein-like substances (Tryptophan-like and tyrosine-like) within the DOM to help characterise and quantify the extent of contamination by effluents from different sources (Hudson et al., 2007). Fulvic- like and humic-like substances are derived from the breakdown of plant material (Stedmon et al., 2003), whereas large inputs of tryptophan-like substances are associated with readily biodegradable material from sewage and farm waste slurry (e.g. Baker, 2001). Agricultural diffuse sources such as animal waste are characterised by high protein-like fluorescence with very high ratios of tryptophan-like to fulvic/humic-like fluorescence compared to stream waters (Baker, 2002), these ratios are sensitive enough to characterise inputs from different livestock animals such as pigs and sheep (Baker, 2002).

Currently, the WHO Guidelines for Drinking Water Quality, adopted as standard in many countries, use total coliforms (TCS), or specifically *Escherichia coli* (*E. coli*) a sub group of faecal coliforms, as faecal indicators for the safety of water supplies. In some countries, such as The Netherlands and Denmark, groundwater is abstracted from sand dune systems to supply drinking water, indicating the importance of understanding the fate and occurrence of TCS and *E. coli* within these systems (Smeets et al., 2009).The enumeration of TCS and *E. coli* is also used as an indicator of water quality within the revised bathing water directive; *E. coli* counts greater than 10,000 counts per 100 ml and TCS counts greater than 2,000 counts per 100 ml would fail to meet the required standards in the directive (European Community, 2006). Although the TCS group includes the species *E. coli*, which is generally considered to be specific for faecal contamination, it also includes other genera such as Klebsiella and Citrobacter which are not necessarily of faecal origin and can emanate from alternative organic sources such as decaying plant materials and soils (WHO, 2006).

While some *E. coli* represent enteric pathogens (Savageau, 1983), other strains of this species can grow and maintain populations in the environment if the conditions are suitable (Byappanahalli and Fujioka, 2004). Sources of *E. coli* include septic tanks, sewer lines, wastewater treatment plants, manure spreading on land, livestock and wildlife. These sources also contribute DOM and nutrient inputs. Despite advanced wastewater treatment efforts by water treatment companies, some UK bathing sites do not always produce full compliance with microbial standards (Crowther et al., 2002) due to other diffuse sources within catchments, resulting in a greater proportion of nonconformity due to agriculture. More than 150 different pathogens, associated with both environmental and human health risks can be found in livestock manure which can significantly increase bacterial loading to the subsurface, causing contamination within soils, groundwater and stream water (Gerba and Smith, 2005). The transport time and distance travelled by bacteria reaching the groundwater or streams depend on the rate at which bacteria are released from manure, the presence of preferential pathway networks within soil and the depth to the groundwater (Abuashour et al., 1994, Unc and Goss, 2003). The presence of TCS in surface or groundwater is usually considered evidence of recent faecal contamination, with *E. coli* remaining active for 16-45 days in the subsurface (Taylor et al., 2004).

This study aims to use a combination of inorganic chemistry, DOM fluorescence and culturable *E. coli* and TCS counts to evaluate the potential sources and pathways of nutrients and contamination to a sand dune site designated for its international nature conservation importance, known to be affected by nutrients from the surrounding agricultural land (Rhymes et al. (2014). Building on the previous study, a further year of bi-monthly sampling was carried out to separately assess the

degree of potential contamination and likely sources of contaminants from a) off-site sources entering the site from streams, b) off-site sources entering the site via runoff/overland flow, c) groundwater flowing under the site, and d) on-site sources.

3.3 Materials and methods

3.3.1 Field monitoring strategy

Aberffraw sand dune system is part of an internationally designated conservation site in the European Union Natura network, located on the southwest corner of the island of Anglesey in North Wales, UK (53°11'N, 4°27'W). It is designated for its dune habitats, in particular its dune slack wetlands and the rare plant and invertebrate species they support (Curreli et al., 2013). The site is in a low valley surrounded on three sides by agricultural land. The agricultural land is reseeded and fertilised pasture, used for sheep and cattle grazing, with feed stations on land immediately adjacent to the south-east dune site edge (Fig 3.1). Streams A and B (Fig 3.1) drain this heavily fertilised agricultural and both lead onto the site. Flow in stream A is episodic and flows primarily in winter, compared with the permanent and faster flowing stream B. Annual long term average rainfall at the site is 847mm (Stratford et al., 2013).There are a number of potential pathways by which nutrients and coliforms can enter the site, these include streams and ditches, surface runoff draining agricultural land and flowing onto the site, seepage of nutrients into the groundwater flowing under the site, and on-site sources such as grazing cattle and rabbits. Previous work has previously shown a nitrogen groundwater contamination gradient that extends into the site from the fertilised pastureland on the south east border with groundwater travelling in a south westerly direction (Fig 3.1) (Rhymes et al., 2014). To determine the nature and pathways of the contamination, measurements were made bimonthly for a 12 month period (i.e. 6 sample periods) across streams, ditches, standing surface water and groundwater in dune slacks. Stream samples were collected from two streams (A and B) entering the site (Fig 3.1). Samples were collected from upstream sampling points (A1 & B1) and downstream sampling points (A2 & B2) by dipping a clean collecting container into the surface flow. Groundwater samples within dune slacks were measured from fifteen groundwater monitoring piezometers across the site, installed to 2m depth. Four piezometers (Fig 3.1, triangles) aimed at looking at impacts from surface runoff. The remaining eleven aimed at evaluating potential gradients in the groundwater of water chemistry, natural fluorescence and TCS and *E. coli* abundance with distance from the contamination sources on the south-east edge of the site (Fig 3.1, squares). Samples were collected from the top 10cm of the water table at each well using a sterilised pump and tubing, which was disinfected with Trigene and

flushed three times with deionised water between samples into 250 mL sterile plastic bottles. During periods of inundation, for up to 4 months between November and February (Rhymes et al., 2014), when water tables were above ground level in certain slacks, samples of the standing water above the piezometer were taken. Groundwater depth was measured monthly at each piezometer.For fluorescence and *E. coli* measurements, sampling was conducted for 4 of the 6 sampling periods.

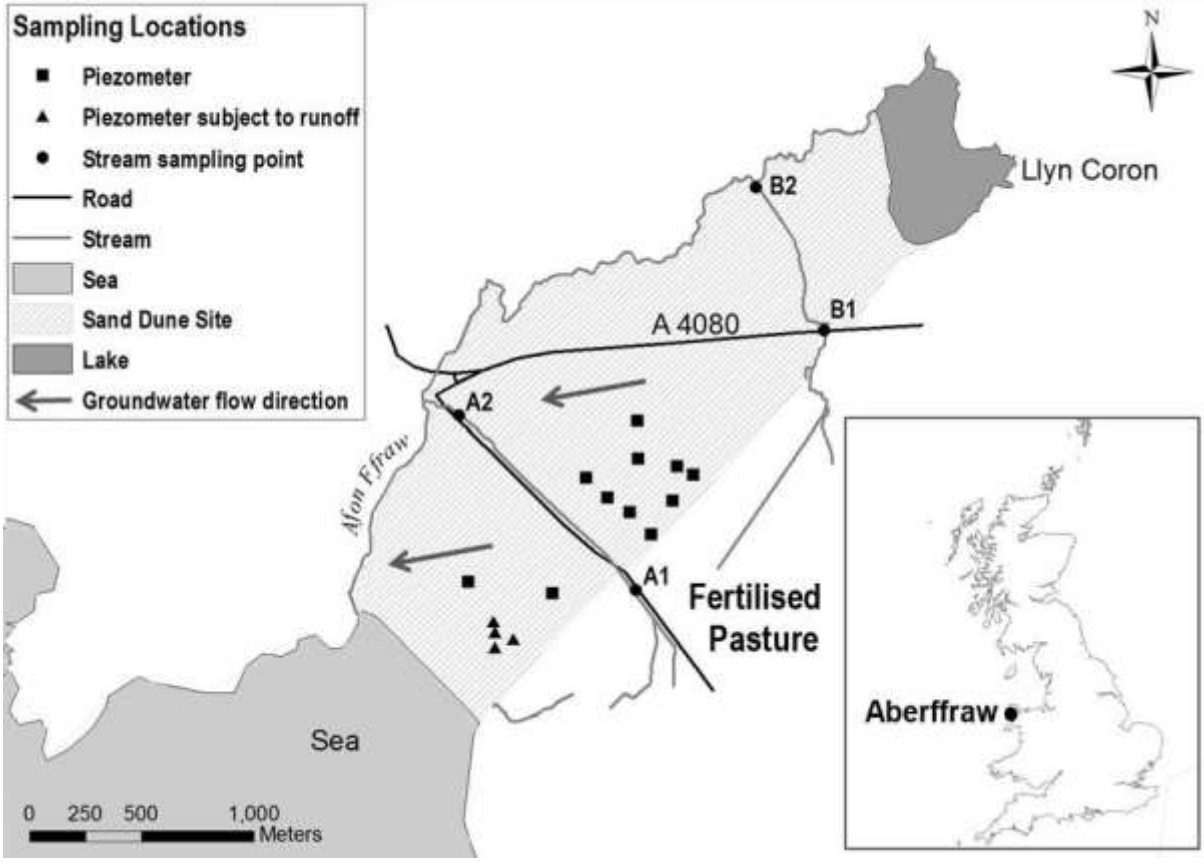


Fig 3.1 Map of Aberffraw dune system showing all piezometer and stream sampling points. Cross-hatched area represents designated site. The surrounding white area is agricultural land, predominantly pastureland. Redrawn from Ordnance Survey.

3.3.2 Water chemistry analysis

Samples from piezometers and streams were stored in darkness at 5°C prior to chemical analysis and analysed within 24 hours. In the laboratory groundwater pH was recorded for each sample which was then filtered through 0.45 µm nylon syringe filter (Avonchem™). Dissolved inorganic anions (fluoride, chloride, nitrite, nitrate, phosphate and sulphate) and cations (sodium, ammonium, potassium, calcium and magnesium) were then quantified on an ion chromatograph (Metrohm, UK Ltd.). Dissolved inorganic nitrogen (DIN) was calculated as the sum of NO3-N, NO2-N and NH4-N. Total nitrogen (TN) and total carbon were analysed by thermal oxidation on a thermalox TOC/TN

analyser. Total inorganic carbon (TIC) was measured within a TIC-reactor on a thermalox. Dissolved organic nitrogen (DON) was calculated by the difference between TN and calculated DIN and Dissolved Organic Carbon (DOC) was calculated by the difference between TCS and TIC.

3.3.3 Fluorescence analysis

All samples were filtered in the field using 0.45um silver membrane filters (Steplitech) and stored in the dark at 5°C prior to analysis. Analysis took place 48 hours after collection at room temperature. Fluorescence measurements were obtained from a spectrophotometer (Variant Cary Eclipse) fitted with a xenon flash lamp using slit widths of 5nm, an integration time of 12.5ms and 700v voltage. Excitation wavelengths were varied from 200 to 400 nm in steps of 5nm and emission wavelengths from 280 to 500 nm in steps of 2 nm. Post processing was carried out using an R script described by Lapworth and Kinniburgh (2009) within the statistical package R v 3.2.0. Absorbance was measured in a 1 cm cuvette on a UV-vis spectrophometer (Varian Cary 50) at 1 nm intervals from 800 to 200 nm. Specific UV absorbance, determined at 254 nm ($SUVA_{254}$) was calculated by dividing absorbance at 254 nm by DOC concentration (Weishaar et al., 2003) and is a measurement of DOC aromaticity. Absorbance measurements were scatter corrected employing the method of Blough et al. (1993). All florescence data was corrected for instrument effects to account for lamp output, and corrected for inner filter effects using the corrected absorbance data (Lakowicz and Geddes, 1991). The data were reported in standard Raman units, which normalises the intensity by the area under the Raman peak between emission wavelengths 380-410 for the excitation wavelength of 348 nm.

3.3.4 Enumeration of TCS *and E. coli*

Water samples were processed within 6 hours of collection. *E. coli* and TCS counts in water samples were determined in duplicate by filtering 20 ml of water through 0.2 µm cellulose nitrate filters (Whatman). Subsequently, cellulose nitrate filters were aseptically transferred onto Harlequin™ *E. coli*/Coliform Medium (Lab M). Culture plates were incubated for 22 hours at 37°C prior to colony counting: TCS generated purple colonies and *E. coli* produced blue colonies Harlequin™ *E. coli*/Coliform Medium.

3.3.5 Statistical analysis

All statistical analysis was performed using Minitab v.16. Differences in stream water chemistry concentrations, fluorescence concentrations and *E. coli* and TCS counts between upstream (A1, B1) and downstream (A2, B2) sampling points in two streams, A and B, were assessed using ANCOVA

(Rutherford, 2001), where the date of sample collection was used as a covariate to account for seasonal variation.

In order to test for statistical differences in chemical, fluorescence and *E. coli* and TCS count variables between streams, runoff and underlying groundwater gradients, annual means of all variables were analysed by grouping piezometers into three classes based on their distance from the south-east site edge (0-150 m, 150-300 m, 300-450 m, excluding piezometers which were subject to run-off), grouping piezometers subject to runoff for March samples alone (see explanation below) and annual mean upstream sampling points from streams A and B (A1 & B1). Statistical tests used analysis of variance. Data that proved not normally distributed (Kolmogrov-Smirnov test) were formed using a Johnson's transformation (Johnson, 1995), which transforms the data to follow a normal distribution using Johnson distribution system.

In order to assess runoff inputs separately from any contributions from on-site sources, samples from piezometers subject to runoff from adjacent pastureland (Fig 3.1 triangles) were compared with all other piezometers (Fig 3.1 squares) for samples collected in March, as this was the only month where all piezometers were subject to groundwater flooding. An ANCOVA (Rutherford, 2001) was carried out on these two groups for all water chemistry, *E. coli* and TCS counts and fluorescent spectroscopy variables, with distance from the south-east site edge as a covariate to account for potential underlying gradients in water chemistry due to other sources. Data that proved not normally distributed (Kolmogorov-Smirnov test) were transformed using a Johnson's transformation (Johnson, 1995).

In order to assess underlying input gradients via the groundwater into the site, whilst accounting for run off inputs, we separately analysed annual means of variables for all piezometers that were not subject to run off (Fig 3.1 squares). Relationships between annual means of all measurements with distance from the south-east site edge were investigated using linear regression. Data that proved not normally distributed (Kolmogorov-Smirnov test) were transformed using a Johnson's transformation (Johnson, 1995).

3.4 Results

When comparing the water chemistry, *E. coli* and TCS counts and fluorescence among the main sources (Table 3.1), nitrate and DIN concentrations in runoff and streams were significantly higher ($F= 10.40$ $df= 4$ $p=0.001$ and $F= 13.76$ $df= 4$ $p= 0.000$) than those found in groundwater samples from the 150-300 m and 300-450 m distance classes. There was no significant difference in nitrate

concentrations between the three distance classes. However, significantly higher concentrations of DIN were observed in the 0-150 m class closest to the south-east site edge compared with the 300-450 m class. *E. coli* counts in the upstream sampling points of streams were significantly higher (F= 68.16 df= 4 p= 0.000) by an order of magnitude than in the runoff samples and in the groundwater at all distance classes (Table 3.1). There were no significant differences between sampling locations for DOC, phosphate, fulvic like, tryptophan like, TRP:FA, $SUVA_{254}$ and TCS. All fluorescent TRP:FA ratios measured within surface waters, groundwater and streams throughout the year did not exceed 1 R.U. and are described as uncontaminated drainage waters (Naden et al., 2010).

Table 3.1 Summary of annual mean water chemistry, *E. coli* and TCS counts (Colony forming units= C.F.U.) and fluorescence concentrations and counts for upstream sampling points for two streams (A and B), mean standing water for flooded slacks subject to runoff in March and for annual mean groundwater and standing water for distance classes (categorised piezometers located 0-150 m, 150-300 m and 300-450 m from within the south east site edge, excluding piezometers subject to run off). Values for each variable are expressed as mean ± standard error. Significant differences among classes are shown in bold; values with the same letter are not significantly different to each other.

Variable		**Stream**	**Groundwater and standing water**			
			Run off	**(Distance from site edge, m)**		
				0-150	**150-300**	**300-450**
Water Chemistry (mg l^{-1})	**Nitrate**	**20.821 ± 9.763 ^{A}**	**7.519 ± 1.556 ^{A}**	**3.197 ± 1.516 ^{AB}**	**0.033 ± 0.019 ^{B}**	**0.018 ± 0.007 ^{B}**
	DIN	**4.720 ± 2.209 ^{A}**	**1.702 ± 0.352 ^{A}**	**0.756 ± 0.346 ^{AC}**	**0.050 ± 0.019 ^{BC}**	**0.017 ± 0.003 ^{B}**
	DON	**0.987 ± 0.459 ^{A}**	**0.658 ± 0.042 ^{AB}**	**0.430 ± 0.076 ^{AB}**	**0.322 ± 0.088 ^{B}**	**0.249 ± 0.034 ^{B}**
	DOC	8.492 ± 13.903	24.600 ± 13.903	14.250 ± 0.966	7.789 ± 4.878	8.602 ± 1.863
	Phosphate	0.750 ± 0.654	0.009 ± 0.003	0.020 ± 0.003	0.012 ± 0.001	0.015 ± 0.005
Bacterial Counts (Log 10 C.F.U. 100 ml^{-1})	***E. coli***	**3.458 ± 0.033 ^{A}**	**0.000 ± 0.000 ^{B}**	**0.151 ± 0.151 ^{B}**	**0.403 ± 0.242 ^{B}**	**0.285 ± 0.133 ^{B}**
	TCS	4.087 ± 0.197	2.559 ± 0.169	2.863 ± 0.083	2.793 ± 0.355	2.641 ± 0.431

Variable		Stream	Groundwater and standing water			
			Run-off	(Distance from site edge, m)		
				0-150	150-300	300-450
Fluorescence (R.U.)	Fulvic like	1.423 ± 0.092	1.491 ± 0.003	1.321 ± 0.048	1.367 ± 0.078	1.251 ± 0.041
	Tryptophan like	1.141 ± 0.030	1.167 ± 0.002	1.114 ± 0.015	1.123 ± 0.024	1.097 ± 0.016
	TRP:FA	0.810 ± 0.036	0.782 ± 0.001	0.844 ± 0.019	0.838 ± 0.027	0.888 ± 0.017
Absorbance (L mg^-1 m^-1)	$SUVA_{254}$	0.043 ± 0.011	0.061 ± 0.046	0.018 ± 0.003	0.020 ± 0.003	0.015 ± 0.005

3.4.1 Stream nutrient and bacterial attenuation

Upstream annual mean nitrate and DIN concentrations are significantly higher (F= 22.14 df= 3 p= 0.000) in stream A than in stream B, however *E. coli* counts are alike for both A and B streams for both upstream and downstream sampling points (Fig 3.2). Annual mean nitrogen concentrations upstream of streams entering the site are high at all stream sampling points (e.g. annual mean 12 mg l^{-1} of nitrate and 2.6 mg l^{-1} of DIN at B1) but are very high in A1, which drains from the south-east site edge into the site, where concentrations reached a maximum of 39 mg l^{-1} of nitrate in January. In stream A, the annual mean concentrations and counts for nitrate, DIN and *E. coli* were significantly higher upstream (A1) than those downstream (A2) (Fig 3.2). By contrast, concentrations of nitrate and DIN in stream B (B1) did not decrease downstream to B2. No significance was found for fluorescence variables for all stream sampling points, TRP:FA mean concentrations were A1= 0.217 ± 0.034 R.U., A2= 0.280 ± 0.042 R.U., B1= 0.457 ± 0.203 R.U. and B2= 0.268 ± 0.042 R.U. Total nitrogen concentrations showed the same significant pattern as nitrate concentrations for all stream sampling points.

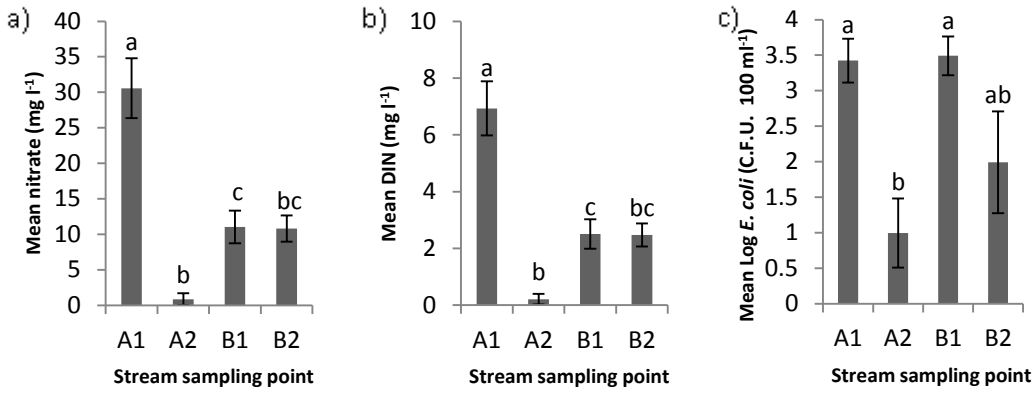


Fig 3.2 Annual mean concentrations for nutrients and *E. coli* counts of two streams (A and B) entering the site at upstream and downstream sampling points, showing: a) Nitrate, b) DIN, c) Log *E. coli* counts. Letters denote significant differences between stream sampling points A1 & B1 – upstream, A2 & B2 downstream – see Fig 3.1.

3.4.2 Run off input

In order to assess the contribution of off-site sources of contamination (cattle feed, overnight dunging, manure or slurry spreading) separately from any contribution by on-site sources (rabbits, dunging of cattle while grazing on-site), slacks only subject to groundwater flooding (Fig 3.1 Squares) were compared with those experiencing groundwater flooding in addition to run off from neighbouring fields (Fig 3.1 Triangles), during a period when both sets of piezometers experienced surface inundation (Table 3.2). Significantly higher concentrations of nitrate and DIN ($F= 10.85$ $df= 1$ $p=0.006$ and $F= 10.25$ $df= 1$ $p = 0.008$) were observed in those slacks exposed to run off (Table 3.2). However, there was no significant difference for DON, DOC, *E. coli* and TCS counts, fluorescent variables and all other variables measured.

Table 3.2 Summary of selected chemical, fluorescent and *E. coli* and TCS count variables for piezometers subject to both run off and groundwater flooding and wells subject to groundwater flooding alone in March. Values for each variable are expressed as mean ± standard error. Significant differences between groups of slacks are shown in bold and denoted by letters.

Variable		**Slacks subject to:**	
		Run off and groundwater flooding	**Groundwater flooding**
Water chemistry (mg l^{-1})	**Nitrate**	**7.519 ± 1.556 [A]**	**0.676 ± 0.386 [B]**
	DIN	**1.702 ± 0.352 [A]**	**0.181 ± 0.0920 [B]**
	DON	0.658 ± 0.042	0.505 ± 0.072
	DOC	11.100 ± 4.700	19.117 ± 2.023
	Phosphate	0.014 ± 0.009	0.024 ± 0.008
Bacterial counts (Log10 C.F.U. ml^{-1})	*E. coli*	0.000 ± 0.000	0.841 ± 0.351
	TCS	3.036 ± 0.301	3.370 ± 0.086
Fluorescence spectroscopy (R.U.)	Fulvic like	1.491 ± 0.003	1.515 ± 0.074
	Tryptophan like	1.167 ± 0.002	1.172 ± 0.023
	TRP:FA	0.782 ± 0.001	0.785 ± 0.024
Absorbance (L mg^-1 m^-1)	$SUVA_{254}$	0.061 ± 0.046	0.018 ± 0.003

3.4.3 Underlying gradients of nitrogen input via groundwater and on-site inputs

In order to assess underlying input gradients via the groundwater into the site, whilst accounting for run off inputs, we separately analysed annual means of variables for all piezometers that were not subject to run off against distance from potential source area at the south-east site edge (Fig 3.3). There were significant gradients of declining nitrate, DIN and DON concentrations into the site, away from their likely source at the south-east site edge (Significant negative regression Fig 3.3 a) Nitrate df= 1 p= 0.029 R^2= 0.391 Coef = -0.005, b) DON df= 1 p= 0.014 R^2= 0.505 Coef= -0.001 and c) DIN df=

1 p= 0.002 R^2= 0.143 Coef= -0.004), with nitrate and DIN decreasing very strongly. A trend of linear decline was apparent for DOC concentrations but this was not significant (Fig 3.3 d). Figure 3.4 shows the spatial pattern of *E. coli* and TCS counts and tryptophan-like and fulvic-like fluorescence. Counts of *E. coli* (Fig 3.3 e and 3.4 b) and TCS (Fig 3.4 a) observed across the site showed no correlation with distance from the south-east site edge, (Log *E. coli* C.F.U. 100 ml^{-1}; max= 4.6, min= 0.00 and Log TCS C.F.U. 100ml^{-1}; max= 4.85, min= 0.00). Tryptophan like fluorescence and fulvic like fluorescence (Fig 3.3 f & g) also showed no correlation with distance from the south-east south edge. However the TRP:FA ratio (Fig 3.3 h)showed a strong positive significant trend for increasing TRP:FA ratio away from the south-east site edge.

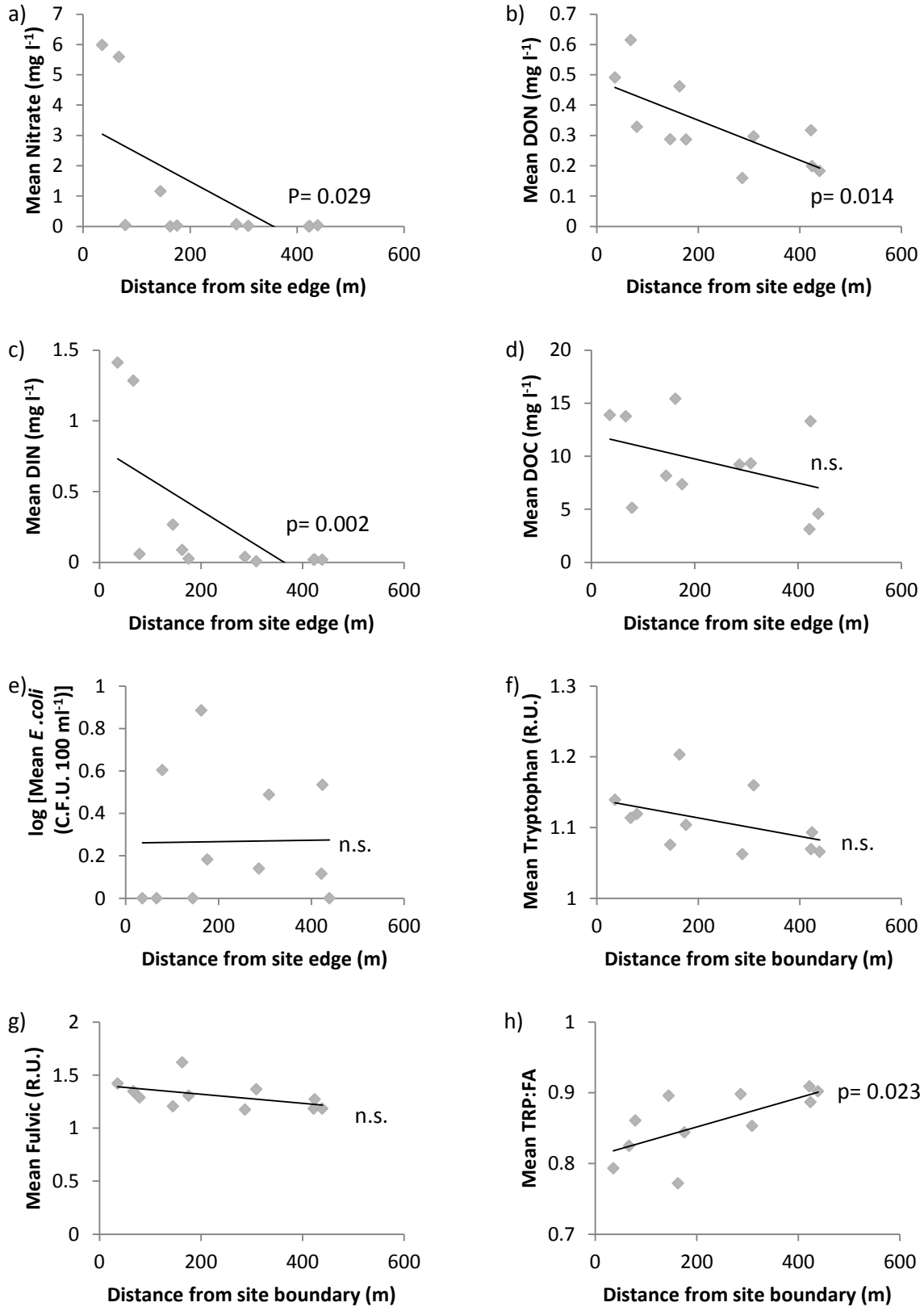


Fig 3.3 Relationships between annual mean groundwater a) Nitrate b) DON c) DIN d) DOC e) *E. coli* f) Tryptophan-like g) Fulvic-like h) TRP:FA with distance from south-east site edge. Trendline for all variables are linear.

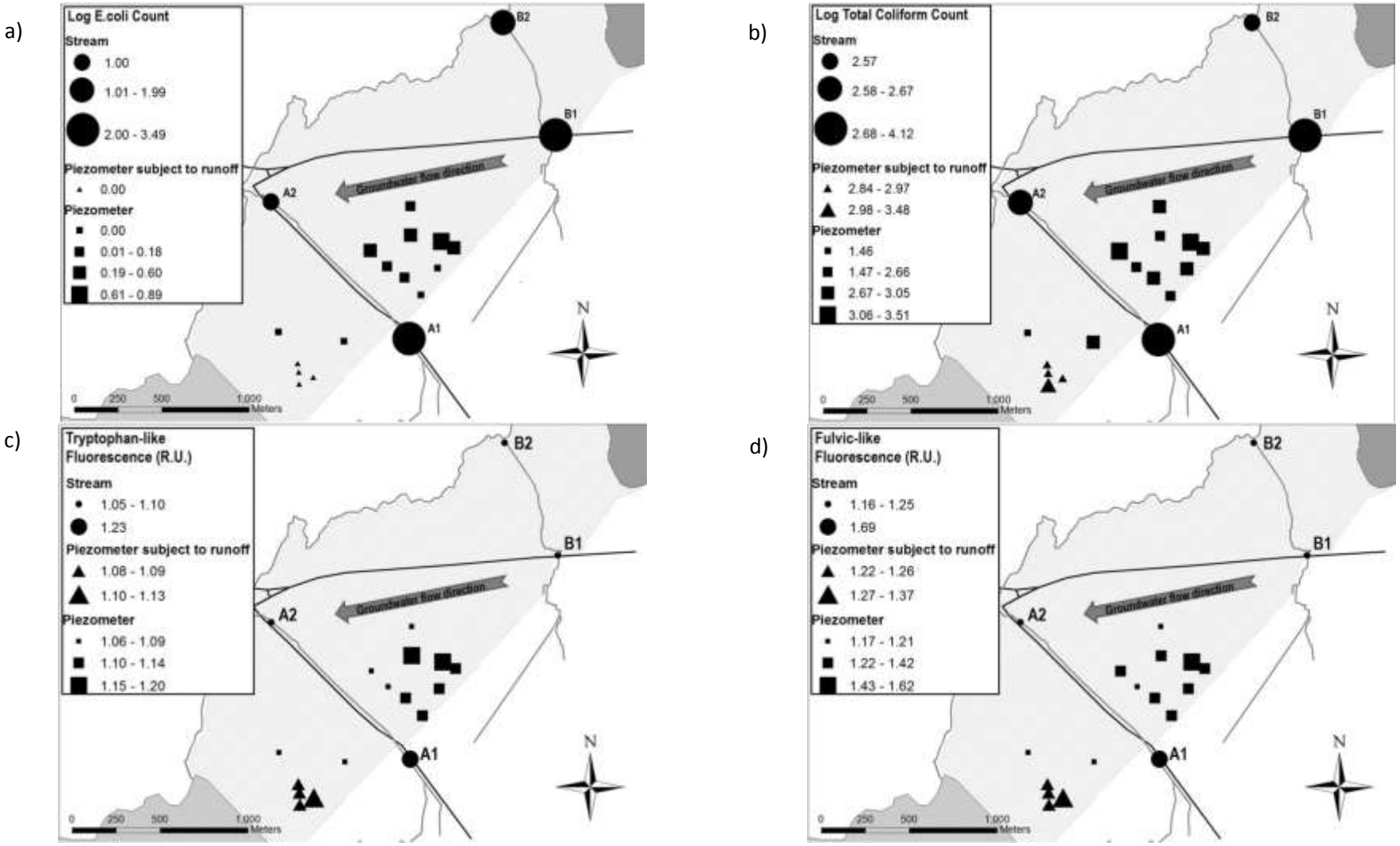


Fig 3.4 Spatial variation of a) Log *E. coli* C.F.U. 100 ml^{-1}, b) Log total coliform C.F.U.100 ml^{-1}, c) tryptophan-like fluorescence (R.U.) and d) fulvic-like fluorescence (R.U) for streams (annual mean), piezometers subject to runoff (March counts and concentrations) and piezometers (annual mean).

3.5 Discussion

In this study we show that a combined analysis of nutrient concentrations, fluorescence and microbial abundance can help identify potential sources and pathways of nutrients and *E. coli* and TCS inputs impacting a wetland site of international nature conservation importance. The multiple pathways and the fate of nutrients and *E. coli* and TCS to the site are summarised in Figure A.2 (See Appendix IV) in the supplementary material.

3.5.1 Off-site sources (fertiliser, cattle dung, slurry and fertilisers applied to fields)

Streams

Streams A and B have similar annual mean *E. coli* abundances as they enter the site, suggesting that both streams have similar faecal inputs from grazers on adjacent pastureland. By contrast, nitrate concentrations in Stream A, which drains the pastureland and flows onto the sand dune site, are significantly higher than those in stream B and previous studies have shown they can exceed the 50 mg l^{-1} threshold for designation of a nitrate vulnerable zone by the UK Environment Agency (Rhymes et al., 2014, Environment Agency, 2012). This implies that stream A has additional N inputs compared to stream B, these are likely to be from fertilisers leaching from the steep sloped pastureland adjoining the stream. This contrast with the fluorescence results where the lack of significant difference in TRP:FA ratio between the two streams suggesting a common contamination source from animal dung, with differences in nutrient loading due to fertiliser inputs only.

The attenuation of nitrate concentrations in stream A is probably caused by a combination of processes, including in-stream microbial denitrification, plant uptake, seepage through the river bank, dilution during high groundwater levels and transient storage (Mulholland et al., 2008). The lack of nutrient attenuation in stream B may be due to the absence of nitrophilous species such as *Phragmites australis* (Stamati et al., 2010) and the faster stream flow in stream B which reduces the water-sediment contact (Peterson et al., 2001) and reduces the ability for microbes to assimilate nitrogen from the water column (Grimm and Fisher, 1989). Similarly *E. coli* counts decrease from upstream to downstream sampling points in stream A but not stream B. Studies have shown that within sediment there are higher populations of TCS than the overlaying water (Smith et al., 2008) as sediments serve as a hospitable environment for bacterial survival due to the availability of organic matter (Jamieson et al., 2005) suggesting that *E. coli* may be being deposited into the stream and incorporated within the sediment. Subsequently during storm events bacteria can be re-suspended into the water column and continue to flow downstream (Jeng et al., 2005), posing a potential threat

to bathing waters on the sandy beach at Aberffraw at the mouth of the river Ffraw since mean *E. coli* colonies within both streams exceed 2,000 colonies per 100 ml which would fail to meet the required standards for the EU Bathing Water Directive (2006).

Surface runoff

The nature of the nitrate contamination contributing via runoff is also likely to be due to applied fertilisers on the south-east pastureland, rather than nutrients from slurry and dung. Nitrate concentrations are eleven times higher in slacks subject to run off and groundwater flooding compared to piezometers subject to groundwater flooding alone, whereas the TRP:FA fluorescence ratios were <1 R.U. which are described as uncontaminated drainage waters compared to described slurry TRP:FA fluorescence ranging from 2-5 R.U. (Naden et al., 2010). In support of this, negligible *E. coli* and TCS counts were observed in slacks subject to runoff which would have been expected if the contamination resulted from slurry application (Thurston-Enriquez et al., 2005). Surface runoff events are sporadic as they are caused by heavy periods of rainfall, nevertheless while nutrient concentrations are high during runoff events and in the subsequent standing surface water, the concentrations in groundwater return to low concentrations once the runoff ceases. This may be a result of denitrification caused by the increased availability of nitrate and anaerobic conditions (Mulvaney et al., 1997), or due to plant and microbial uptake highlighting the function of dune systems in filtering nutrients. However, the initial nitrate and DIN concentrations in the standing water are much higher than the levels of 0.2 mg DIN l^{-1} above which biological effects have been determined previously at the site (Rhymes et al. 2014), suggesting adverse impacts on the site due to nutrients from this source. In addition, the total flux of nitrogen entering the site as a result of these runoff events is also unknown. Calculating this input may help harmonise dose-related critical load approaches to damage (Bobbink and Hettelingh, 2010) with concentration based methods used in aquatic systems (Camargo and Alonso, 2006).

Groundwater

Nutrients, likely to be from ammonium nitrate fertiliser, are entering the site by leaching through sandy agricultural soils with a low water holding capacity (Skiba and Wainwright, 1984), and subsequently flowing under the site via the groundwater. As a result a nitrate, DIN and DON groundwater gradient was found independently of runoff influence, confirming that the gradients of elevated nutrients in groundwater and soils observed in an earlier study were not due to surface flooding (Rhymes et al., 2014). There was no gradient in *E. coli* and TCS counts within the groundwater under the site, suggesting that the higher concentrations of *E. coli* and TCS originating

in the south-east pasture and observed in the streams are probably filtered out by the sandy soils during recharge transit in the subsurface before reaching the site (Price et al., 2013).

3.5.2 On-site sources (rabbits, cattle dung)

E. coli and TCS were found across the site at low levels, but showed no relationship with the distance from the south-east site edge. Similarly, the tryptophan like fluorescence showed no relationship with the distance from the south-east site edge. This suggests that the main source of groundwater *E. coli*, and TCS, derive from on-site cattle and rabbits. Therefore, in addition to streambed seepage, run off and underlying groundwater nutrient inputs, there are nutrient and *E. coli* and TCS inputs from on-site grazers such as cattle and rabbits. Despite these small-scale on-site inputs, all piezometers across the site meet the mandatory bathing water directive standards of 2,000 *E. coli* C.F.U. 100 ml^{-1}.

3.6 Conclusions

The combination of chemical, fluorescent and microbial techniques has helped identify potential nutrient sources from fertilisers and grazers (Baker, 2002). The findings of this study suggest nutrients are being attenuated and processed within the site thereby providing a valuable ecosystem service. However at the same time, the influx of nutrients is likely to have an adverse effect on the dune slack ecology, with impacts on plant community composition (Rhymes et al., 2014). The analysis of surface waters, slacks and piezometers across the site has allowed the differentiation between the input pathways of streams, run off events, underlying groundwater nutrient gradients and on-site grazing inputs. While the study was able to distinguish multiple pathways, the full potential of the techniques to differentiate between livestock sources (e.g. sheep, cattle, pigs) was not explored in this study. This combination of techniques provides an approach which could allow for a detailed understanding of nutrient contamination sources and pathways relatively cheaply. Such information is key for designing successful management plans to reduce the inputs of contaminants which might be having detrimental effects on sites of conservational value. It could also be implemented for other applications such as tracking faecal sources within bathing waters and fisheries zones, as currently the standard enumeration of faecal indicator bacteria (FIB) does not distinguish between human or other animal sources of contamination, and methods that do so are expensive (Field and Samadpour, 2007).

3.7 References

ABUASHOUR, J., JOY, D. M., LEE, H., WHITELEY, H. R. & ZELIN, S. 1994. Transport of microorganisms through soil. *Water Air and Soil Pollution,* 75**,** 141-158.

BAKER, A. 2001. Fluorescence excitation-emission matrix characterization of some sewage-impacted rivers. *Environmental Science & Technology,* 35**,** 948-953.

BAKER, A. 2002. Fluorescence properties of some farm wastes: implications for water quality monitoring. *Water Research,* 36**,** 189-195.

BLOUGH, N. V., ZAFIRIOU, O. C. & BONILLA, J. 1993. Optical-absorption spectra of waters from the orinoco river outflow - terrestrial input of colored organic-matter to the caribbean. *Journal of Geophysical Research-Oceans,* 98**,** 2271-2278.

BOBBINK, R. & HETTELINGH, J.-P. Review and revision of empirical critical loads and dose-response relationships. Proceedings of an expert workshop, Noordwijkerhout, 2010. 23-25.

BYAPPANAHALLI, M. & FUJIOKA, R. 2004. Indigenous soil bacteria and low moisture may limit but allow faecal bacteria to multiply and become a minor population in tropical soils. *Water Science and Technology,* 50**,** 27-32.

CAMARGO, J. A. & ALONSO, A. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International,* 32**,** 831-849.

COMMUNITY, E. 2006. Directive 2006/7/EC of the European Parliament and of the Council of 15 February (2006) concerning the management of bathing water quality and repealing Directive 76/160/EEC. *Official Journal of the European Union*, 4.3.2006, L64/37.

CROWTHER, J., KAY, D. & WYER, M. D. 2002. Faecal-indicator concentrations in waters draining lowland pastoral catchments in the UK: relationships with land use and farming practices. *Water Research,* 36**,** 1725-1734.

CURRELI, A., WALLACE, H., FREEMAN, C., HOLLINGHAM, M., STRATFORD, C., JOHNSON, H. & JONES, L. 2013. Eco-hydrological requirements of dune slack vegetation and the implications of climate change. *Science of the Total Environment,* 443**,** 910-919.

ENVIRONMENT AGENCY. 2012. Method statement for nitrate vulnerable zone review. Surface waters environment agency report to Defra and Welsh Government — supporting paper for the implementation of the nitrates directive 2013–2016.

FIELD, C. D., DISE, N. B., R.J., P., BRITTON, A. J., EMMETT, B. A., HELLIWELL, R. C., HUGHES, S., JONES, L., LEES, S., LEAKE, J. R., LEITH, I. D., PHOENIX, G. K., POWER, S. A., SHEPPARD, L. J., SOUTHON, G. E., STEVENS, C. J. & CAPORN, S. J. M. 2014. The role of nitrogen deposition in widespread plant community change across semi-natural habitats. *Ecosystems*.

FIELD, K. G. & SAMADPOUR, M. 2007. Fecal source tracking, the indicator paradigm, and managing water quality. *Water Research,* 41**,** 3517-3538.

FOWLER, D., O'DONOGHUE, M., MULLER, J., SMITH, R., DRAGOSITS, U., SKIBA, U., SUTTON, M. & BRIMBLECOMBE, P. 2005. A chronology of nitrogen deposition in the UK between 1900 and 2000. *Water, Air, & Soil Pollution: Focus,* 4**,** 9-23.

GALLOWAY, J. N. & COWLING, E. B. 2002. Reactive nitrogen and the world: 200 years of change. *Ambio,* 31**,** 64-71.

GERBA, C. P. & SMITH, J. E. 2005. Sources of pathogenic microorganisms and their fate during land application of wastes. *Journal of Environmental Quality,* 34**,** 42-48.

GRIMM, N. B. & FISHER, S. G. 1989. Stability of periphyton and macroinvertebrates to disturbance by flash floods in a desert stream. *Journal of the North American Benthological Society,* 8**,** 293-307.

HOODA, P. S., EDWARDS, A. C., ANDERSON, H. A. & MILLER, A. 2000. A review of water quality concerns in livestock farming areas. *Science of the Total Environment,* 250**,** 143-167.

HUDSON, N., BAKER, A. & REYNOLDS, D. 2007. Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters - A review. *River Research and Applications,* 23**,** 631-649.

JAMIESON, R., JOY, D. M., LEE, H., KOSTASCHUK, R. & GORDON, R. 2005. Transport and deposition of sediment-associated *Escherichia coli* in natural streams. *Water Research,* 39**,** 2665-2675.

JENG, H. W. C., ENGLAND, A. J. & BRADFORD, H. B. 2005. Indicator organisms associated with stormwater suspended particles and estuarine sediment. *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering,* 40**,** 779-791.

JOHNSON, D. H. 1995. Statistical sirens: the allure of nonparametrics. *Ecology*, 1998-2000.

JONES, L., NIZAM, M., REYNOLDS, B., BAREHAM, S. & OXLEY, E. 2013. Upwind impacts of ammonia from an intensive poultry unit. *Environmental Pollution,* 180**,** 221-228.

KAY, D., CROWTHER, J., FEWTRELL, L., FRANCIS, C. A., HOPKINS, M., KAY, C., MCDONALD, A. T., STAPLETON, C. M., WATKINS, J., WILKINSON, J. & WYER, M. D. 2008. Quantification and control of microbial pollution from agriculture: a new policy challenge? *Environmental Science & Policy,* 11**,** 171-184.

LAKOWICZ, J. R. & GEDDES, C. D. 1991. Topics in fluorescence spectroscopy. Plenum Press: New York, 1991; Chapter 5.

LAPWORTH, D. J. & KINNIBURGH, D. G. 2009. An R script for visualising and analysing fluorescence excitation-emission matrices (EEMs). *Computers & Geosciences,* 35**,** 2160-2163.

MULHOLLAND, P. J., HELTON, A. M., POOLE, G. C., HALL, R. O., HAMILTON, S. K., PETERSON, B. J., TANK, J. L., ASHKENAS, L. R., COOPER, L. W., DAHM, C. N., DODDS, W. K., FINDLAY, S. E. G., GREGORY, S. V., GRIMM, N. B., JOHNSON, S. L., MCDOWELL, W. H., MEYER, J. L., VALETT, H. M., WEBSTER, J. R., ARANGO, C. P., BEAULIEU, J. J., BERNOT, M. J., BURGIN, A. J., CRENSHAW, C. L., JOHNSON, L. T., NIEDERLEHNER, B. R., O'BRIEN, J. M., POTTER, J. D., SHEIBLEY, R. W., SOBOTA, D. J. & THOMAS, S. M. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature,* 452**,** 202-U46.

MULVANEY, R. L., KHAN, S. A. & MULVANEY, C. S. 1997. Nitrogen fertilizers promote denitrification. *Biology and Fertility of Soils,* 24**,** 211-220.

NADEN, P. S., OLD, G. H., ELIOT-LAIZE, C., GRANGER, S. J., HAWKINS, J. M. B., BOL, R. & HAYGARTH, P. 2010. Assessment of natural fluorescence as a tracer of diffuse agricultural pollution from slurry spreading on intensely-farmed grasslands. *Water Research,* 44**,** 1701-1712.

NOVOTNY, V. 1999. Diffuse pollution from agriculture - A worldwide outlook. *Water Science and Technology,* 39**,** 1-13.

OAKES, D. B., YOUNG, C. P. & FOSTER, S. S. D. 1981. The effects of farming practices on groundwater quality in the United-Kingdom. *Science of the Total Environment,* 21**,** 17-30.

OLD, G. H., NADEN, P. S., GRANGER, S. J., BILOTTA, G. S., BRAZIER, R. E., MACLEOD, C. J. A., KRUEGER, T., BOL, R., HAWKINS, J. M. B., HAYGARTH, P. & FREER, J. 2012. A novel application of natural

fluorescence to understand the sources and transport pathways of pollutants from livestock farming in small headwater catchments. *Science of the Total Environment,* 417**,** 169-182.

PETERSON, B. J., WOLLHEIM, W. M., MULHOLLAND, P. J., WEBSTER, J. R., MEYER, J. L., TANK, J. L., MARTI, E., BOWDEN, W. B., VALETT, H. M., HERSHEY, A. E., MCDOWELL, W. H., DODDS, W. K., HAMILTON, S. K., GREGORY, S. & MORRALL, D. D. 2001. Control of nitrogen export from watersheds by headwater streams. *Science,* 292**,** 86-90.

PLASSMANN, K., BROWN, N., JONES, M. L. M. & EDWARDS-JONES, G. 2008. Can atmospheric input of nitrogen affect seed bank dynamics in habitats of conservation interest? The case of dune slacks. *Applied Vegetation Science,* 11**,** 413-420.

PRICE, W. D., BURCHELL, M. R., HUNT, W. F. & CHESCHEIR, G. M. 2013. Long-term study of dune infiltration systems to treat coastal stormwater runoff for fecal bacteria. *Ecological Engineering,* 52**,** 1-11.

RHYMES, J., WALLACE, H., FENNER, N. & JONES, L. 2014. Evidence for sensitivity of dune wetlands to groundwater nutrients. *Science of the Total Environment,* 490C**,** 106-113.

RUTHERFORD, A. 2001. Introducingn ANOVA and ANCOVA: a GLM approach. *Sage.*

SAVAGEAU, M. A. 1983. *Escherichia-Coli* habitats, cell-types, and molecular mechanisms of gene-control. *American Naturalist,* 122**,** 732-744.

SKIBA, U. & WAINWRIGHT, M. 1984. Nitrogen transformation in coastal sands and dune soils. *Journal of Arid Environments,* 7**,** 1-8.

SMEETS, P., MEDEMA, G. & VAN DIJK, J. 2009. The Dutch secret: how to provide safe drinking water without chlorine in the Netherlands. *Drinking Water Engineering and Science,* 2**,** 1-14.

SMITH, J., EDWARDS, J., HILGER, H. & STECK, T. R. 2008. Sediment can be a reservoir for coliform bacteria released into streams. *The Journal of general and applied microbiology,* 54**,** 173-179.

STAMATI, F. E., CHALKIAS, N., MORAETIS, D. & NIKOLAIDIS, N. P. 2010. Natural attenuation of nutrients in a mediterranean drainage canal. *Journal of Environmental Monitoring,* 12**,** 164-171.

STEDMON, C. A., MARKAGER, S. & BRO, R. 2003. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. *Marine Chemistry,* 82**,** 239-254.

STRATFORD, C. J., ROBINS, N. S., CLARKE, D., JONES, L. & WEAVER, G. 2013. An ecohydrological review of dune slacks on the west coast of England and Wales. *Ecohydrology,* 6**,** 162-171.

TAYLOR, R., CRONIN, A., PEDLEY, S., BARKER, J. & ATKINSON, T. 2004. The implications of groundwater velocity variations on microbial transport and wellhead protection - review of field evidence. *Fems Microbiology Ecology,* 49**,** 17-26.

THURSTON-ENRIQUEZ, J. A., GILLEY, J. E. & EGHBALL, B. 2005. Microbial quality of runoff following land application of cattle manure and swine slurry. *Journal of Water and Health,* 3**,** 157-171.

UNC, A. & GOSS, M. J. 2003. Movement of faecal bacteria through the vadose zone. *Water Air and Soil Pollution,* 149**,** 327-337.

VADAS, P. A., HARMEL, R. D. & KLEINMAN, P. J. A. 2007. Transformations of soil and manure phosphorus after surface application of manure to field plots. *Nutrient Cycling in Agroecosystems,* 77**,** 83-99.

WEISHAAR, J. L., AIKEN, G. R., BERGAMASCHI, B. A., FRAM, M. S., FUJII, R. & MOPPER, K. 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental Science & Technology,* 37**,** 4702-4708.

WHO 2006. Guidelines for drinking-water quality: First addendum to volume 1, Recommendations, World Health Organization.

WITHERS, P. J. A., JARVIE, H. P., HODGKINSON, R. A., PALMER-FELGATE, E. J., BATES, A., NEAL, M., HOWELLS, R., WITHERS, C. M. & WICKHAM, H. D. 2009. Characterization of phosphorus sources in rural watersheds. *Journal of Environmental Quality,* 38**,** 1998-2011.

CHAPTER 4: **Groundwater nutrients and water levels alter nitrogen and carbon processing in dune slack soils**

J. Rhymes [1], L. Jones [2], H. Wallace [3], T. Jones [1], C. Dunn [1], N. Fenner [1].

[1]Bangor University, UK; [2]Centre for ecology and hydrology, UK; [3] Ecological surveys (Bangor), UK

Author Contributions: The chapter was written by Jennifer Rhymes. Laurence Jones, Nathalie Fenner and Hilary Wallace provided advice on the experimental design and gave editorial suggestions. Christian Dunn and Timothy Jones provided technical laboratory advice.

4.1 Abstract

Dune slacks are seasonal wetlands which experience considerable fluctuation in water table depth. They are of biological importance because they are high in biodiversity. Dune slacks are subject to multiple threats such as eutrophication and lowered water tables from climate change and water abstraction. The biological effects caused by the interactions of these pressures are poorly understood and so here we used a mesocosm and laboratory assays to measure the impact of groundwater nitrogen contamination, lowered water table and the synergistic effects of both on soil microbial processes and greenhouse gas emissions. This study showed that N_2O emissions occurred for longer durations within dune slack soils subject to higher concentrations of groundwater nitrogen contamination. Lowered water tables however showed a reduction in denitrification and indeed we found increased soil nitrogen content from just a 10 cm decrease in water table depth. The results from extracellular enzyme assays, suggest that decomposition rates increase within drier soils given the increase in β-glucosidase activity, with further sensitivity to groundwater nitrogen contamination given the increase in phenol oxidase activity. Dune slack soils with a 10 cm higher water table however, are subject to significantly higher rates of methanogenesis soil processes, with CH_4 emissions nearly 5 times higher in wetter soils. Our findings demonstrate that dune slacks are sensitive to both groundwater nitrogen contamination and lowered water tables, whilst synergistically the biological impacts from lowered water tables are likely to be intensified from groundwater nitrogen contamination

4.2 Introduction

Wet dune slacks are seasonal wetlands occupying low-lying areas within a sand dune system which support a diverse flora of conservation value (Grootjans et al., 2004). They are subject to seasonal variations in water tables, with water tables highest during the winter and falling during the summer (van der Laan, 1979). Water table depth can vary significantly from year to year (Stratford et al., 2013, Robins and Jones, 2013). These fluctuations play a key role in controlling nutrient and carbon processes within dune slack soils, conserving the low nutrient status required by dune slack species (Berendse et al., 1998). Dune slacks are subject to multiple threats such as eutrophication, and lowered water tables from climate change and water abstraction. It is therefore of importance to identify the effect of predicted increases in nitrogen availability (Galloway and Cowling, 2002) and water table lowering (Clarke and Ayutthaya, 2010) on dune slack soil biogeochemistry.

The impacts of atmospheric nitrogen deposition on dry dune habitats has been investigated within various studies (Plassmann et al., 2009, Remke et al., 2009, Jones et al., 2013). Few studies however, have investigated the impacts of nitrogen inputs on dune slack ecology (Willis et al., 1959, Plassmann et al., 2010, Rhymes et al., 2014) and only one of these provides evidence of impacts from groundwater dissolved inorganic nitrogen (DIN) contamination, at concentrations as low as 0.2 mg l^{-1} (Rhymes et al., 2014). Denitrification is important in regulating nitrogen concentrations within wetland ecosystems (Camargo and Alonso, 2006), including dune slack habitats that are vulnerable to nitrogen contamination (Seitzinger et al., 2006). Denitrification rates are controlled by multiple factors such as soil moisture content (Hefting et al., 2004), nitrate concentrations (Merrill and Zak, 1992) and soil O_2 levels (Burgin et al., 2010). During periods when dune slack soils are waterlogged, the favoured anaerobic conditions for denitrification are met (Berendse et al., 1998) and soil nitrate is reduced to gaseous nitrogen products (N_2, N_2O and NO) by microbial processes (Knowles, 1982). Under complete anaerobic conditions, N_2 is the end product and where oxygen levels are higher the denitrification reaction stops with the formation of NO_x (Brady and Weil, 2002), N_2O production however tends to be as a result of low soil pH or high nitrate concentrations. The measurement of N_2O within wetland studies is therefore often used as an indicator of soil denitrification (Bernot et al., 2003, DeLaune and Jugsujinda, 2003), and will be utilised within this study as it is difficult to measure N_2 production against high atmospheric N_2 background concentrations (Groffman et al., 2006).

Decomposition rates are controlled by temperature and soil moisture content, with slower decomposition and subsequent increases in soil development within cooler and wetter soils (Jones

et al., 2008). In systems which are N limited, increases in nitrogen inputs tend to increase decomposition rates. Decomposition can be measured by soil respiration, an indicator of aerobic microbial decomposition, and by methane emissions, an indicator of anaerobic microbial decomposition of soil organic matter (Whalen, 2005).

The measurement of extracellular enzyme activities within soils can further quantify biogeochemical processes linked to nutrient and carbon cycling, allowing an understanding of microbial ecology within different environmental conditions. Extracellular enzyme activities and their response to environmental change have been investigated in multiple soil types (Henry, 2012), however, to our knowledge these measurements have not been carried out within dune slack soils. The hydrolase enzyme N-acetyl-β-glucosaminidase (NAG) is responsible for the breakdown of chitin, an essential process in nitrogen cycling (Kang et al., 2005) and β-glucosidase (BG) for the degradation of cellulose to glucose, providing one of the most important sources of labile carbon for soil microbes (Deng, 2011). Phenol oxidase enzyme (POX) degrades phenolic material (McLatchey and Reddy, 1998). Even though this is not involved with nitrogen cycling directly, the build-up of phenolics from low POX activity can mediate the activity of hydrolase enzymes, such as NAG (Freeman et al., 2001). The measurement of POX therefore helps the interpretation of NAG and BG responses to nitrogen contamination and climate change.

This study aimed to investigate the impacts of predicted increases in nitrogen availability (Camargo and Alonso, 2006), drier conditions (Clarke and Ayutthaya, 2010) and their interaction on dune slack biogeochemistry. We tested the following research questions using analysis of soil chemistry, extracellular enzyme activities and greenhouse gas measurements; does groundwater nitrogen contamination increase dune slack soil denitrification? Does groundwater nitrogen contamination inhibit carbon soil processes? Do lowered water tables decrease denitrification? Do lowered water tables increase soil decomposition?

4.3 Methods

Dune slack soil was collected from a previously uncontaminated *Salix repens-Calliergon cuspidatum stellatum* community dune slack at Aberffraw, identified by the presence of pristine vegetation communities and groundwater NO_3 concentration (Rhymes et al., 2014) (Anglesey, North Wales, UK, 53°11'N, 4°27'W) and separated into two soil types; an organic top 10 cm layer and mineral sand from depth range -10 to -50 cm. Roots were removed by hand and soil was homogenised with a cement mixer and used for two complementary experimental designs (both described below);

microcosms were used to allow close control of potentially confounding factors, whilst mesocosms represent more natural conditions and ran for a longer duration.

4.3.1 Experimental designs

Microcosm experiment

Microcosms were prepared in 50ml falcon tubes (Corning inc.) wrapped in foil with 15 g of the organic homogenised dune slack soil. Once arranged the microcosms were left to equilibrate for 24 hours and kept in complete darkness at 18 °C for the duration of the experiment. Groundwater treatments were produced by adding calculated volumes of ammonium nitrate to groundwater collected from a dune slack with low nitrogen background concentrations (0.075mg l^{-1} of DIN), to produce concentrations of 0, 1, 3, and 10 mg l^{-1} of DIN. 5 ml of treatment was then added to 15 replicates for each treatment (10 replicates for soil sampling and 5 replicates for gas sampling). Microcosms were sampled for enzyme activity 24 and 74 hours after treatment addition and gas samples were collected 1, 4, 8, 24, 48 and 72 hours after treatment was added.

Mesocosm experiment

Each mesocosm was constructed with plastic pipe (50 cm height and 16 cm diameter) with a mesh-lined perforated plastic base attached to the bottom for drainage (Fig 4.1). The first 42 cm was filled with mineral sand with no organic matter (described above), whilst the top 8 cm was filled with homogenised organic matter. Each mesocosm was planted with four typical dune slack species (2 sedges and 2 forb species); one specimen each of *Carex arenaria*, *Carex flacca*, *Leontodon autumnalis* and *Prunella vulgaris*. The mesocosms were then placed into individual buckets filled with artificial groundwater and treatments (see details below). Holes within the side of the buckets were used to control water table regimes and were attached to plastic tubing to collect any overflow. Black plastic was used to cover the opening of the bucket to exclude light, which did not permit rainfall to mix directly into the groundwater and to avoid water loss through evaporation. The outer part of the mesocosms, buckets and outlet bottles were wrapped in foil to minimise absorption of the sun's heat.

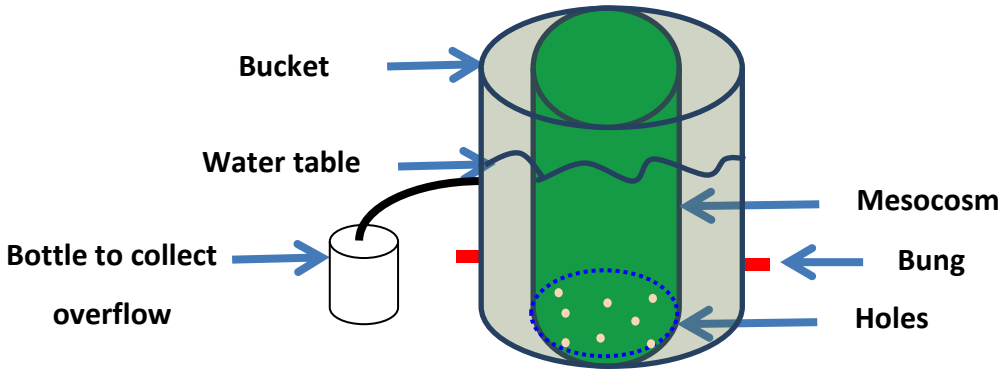


Fig 4.1 Diagram of a constructed mesocosm.

This experiment ran from October 2013 to July 2014 in Bangor, North Wales, UK (53°13'32.0"N, 4°07'55.1"W) and involved six DIN groundwater treatments; control (0.0 mg l^{-1} of DIN), low (0.2 mg l^{-1} of DIN) and high (10 mg l^{-1} of DIN) in factorial combination with a wet or dry hydrological regime, each with 8 replicates. Wet hydrological regimes were altered from -10 cm water table depth in the winter months to -30 cm in the summer months, whilst the dry hydrological treatments were altered to consistently be 10 cm lower than the wet treatment. The artificial groundwater was synthesised by adding the listed compounds (Table 4.1) to 20L of de-ionised water, mimicking concentrations of cations, anions and the low pH measured at Aberffraw previously (Rhymes et al., 2014). Due to rainfall and evaporation water tables fluctuated in line with typical hydrological regimes in the field, although were unable to flood. On the 1st of July 2014 two litres of artificial groundwater (Table 4.1) was added to each mesocosm to avoid causing drought conditions within the mesocosms due to a long period without rainfall. Nitrogen treatments were maintained monthly by calculating amounts of ammonium nitrate required to meet targeted DIN treatment concentrations.

Table 4.1 Artificial groundwater recipe; compound weights added to 20L de-ionised water

Compound	Weight (g)
$CaCO_3$	0.941
$CaCl_2.6H_2O$	7.541
$MgSO_4.7H_2O$	0.370
$MgCl_2.6H_2O$	0.996
KCl	0.089
$NaHCO_3$	6.082

4.3.2 Soil sampling

Ten replicate microcosms were sampled for determining enzyme activity, 5 randomly selected replicates were utilised at 24 and another 5 (Total= 10) at 74 hours after treatment for each treatment. Soil from each microcosm were homogenised with a spatula and weighed out. Mesocosm soil samples were collected over a 3 day period from 22nd to the 24th July 2014, from 6 replicates of each treatment (2 replicates per treatment per day), where 4 cm length x 6 cm width x 8 cm height soil samples were taken from the middle of the mesocosm and placed into a sealable plastic bag. Samples were then de-rooted, homogenised by hand and weighed out (see analysis below). Both mesocosm enzyme activity and soil chemistry were analysed immediately after soil collection and preparation.

4.3.3 Gas sampling

Both microcosm and mesocosm gas samples were taken using a 20 cm^3 syringe fitted with a two-way valve (Sigma, Aldrich Ltd.) and a short bevel hypodermic needle then injected into 12 ml evacuated exetainers (Labco Ltd.). Microcosm lids were fitted with a Suba-Seal® rubber septa (Sigma Aldrich Ltd.) and placed onto individual microcosms (5 microcosms per treatment), with gas samples being collected after an hour. This procedure was carried out 1, 4, 8, 24, 48 and 72 hours after the addition of DIN groundwater treatment. Three ambient gas samples were taken prior to enclosing each microcosm with a lid.

Mesocosm gas samples were taken 30 min and 1 hour after attaching an air-tight transparent chamber fitted with a Suba-Seal® rubber septa (Sigma Aldrich Ltd.) (N_2O gas concentrations measured 30 min after incubation and 1 hour for CO_2 and CH_4, see appendix V, A2 for gas linearity). Samples were taken from 4 randomised replicates of each treatment on a winter day (29/01/14) and summer night (21/07/14) and 6 replicates of each treatment for summer days, over 3 days (22^{nd} to $24^{th}$of July, 2 replicates per treatment per day), which coincided with the mesocosm soil sampling (See above). Three ambient samples were taken prior to attaching the chamber.

4.3.4 Laboratory analysis

Soil moisture content and LOI

In both microcosm and mesocosm experiments a sub sample (6-8 g of fresh soil) was weighed, dried at 105 °C and re-weighed to measure moisture content within 24 hours of collection. The samples

were then heated in a furnace at 375 °C for 16 h and re-weighed to calculate organic matter content through loss on ignition (Ball, 1963).

Soil chemistry

A sub sample from the mesocosm experiment was prepared for chemical analysis using a water extraction of 5 g of homogenised soil, mixed with 40 ml ultra-high purity water (1:10 wt/vol) for 24 hours on an orbital shaker (Chantigny, 2003). The solution was then centrifuged for 15 min at 5000 rpm and filtered through 0.45 µm nylon syringe filter (Avonchem ™). Nitrate, nitrite and ammonium were quantified on an ion chromatograph (Metrohm, UK Ltd.). Dissolved inorganic nitrogen (DIN) was calculated as the sum of NO_3-N, NO_2-N and NH_4-N. Total nitrogen (TN) and total carbon were analysed by thermal oxidation on a TOC/TN analyser (Thermalox, UK) whilst total inorganic carbon (TIC) was measured using a TIC-reactor (Thermalox, UK). Dissolved organic nitrogen (DON) was calculated by the difference between TN and calculated DIN.

Enzyme analysis

In both experiments soil samples were assayed for the activity of three extracellular enzymes, the names and functions for each enzyme are listed in table 4.2. Hydrolase enzyme activity (N-acetyl-β-glucosaminidase and β-glucosidase) was measured using 1 g of soil and a modified method from Freeman et al. (1995). Phenol oxidase activity was measured using 1 g of soil and a modified method from Pind et al. (1994). Modifications for both methods are described by Dunn et al. (2014) (It should be noted that complete saturation curves were not explored for dune slack soils and must therefore not be compared with the wider literature however, the technique allows for the comparison of potential enzyme activity across treatments rather than absolute activity). All substrates and soils were incubated at 18 °C for microcosm soils and 16 °C for mesocosm soils (18 °C was the optimal soil temperatures recorded within the field, whilst 16 °C was the recorded temperature during mesocosm soil sample collection).

Table 4.2 MUF-labelled substrates required to measure specified extracellular enzyme activity.

Substrate	Enzyme	Abbreviation	Enzyme commission number	Function
4-MUF N-acetyl-β-glucosaminide	N-acetyl-β-glucosaminidase	NAG	3.2.1.96	Breaks down chitin
4-MUF β-glucopyranoside	β-glucosidase	BG	3.2.1.21	Hydrolyses carbohydrate molecule
L-Dopa	Phenol oxidase	POX	1.10.3.2	Oxidises phenolic compounds

Gas analysis

Gas samples were analysed by gas chromatography using a Varian model 450 gas chromatograph (GC) instrument, equipped with a flame ionisation detector (FID) with a CO_2 to CH_4 catalytic converter (methaniser), to measure concentrations of CO_2 and CH_4 and an electron capture detector (ECD) for N_2O. Two mL of sample gas was injected via a 1041 on-column injector system onto a PoroPak QS (1.83m x 3.18mm) 80/100 column. Methane, CO_2, and N_2O (retention times 1.08, 1.87 and 2.25 minutes respectively) were quantified by comparison of peak area with that of the three standards of known concentration used in the preparation of a standard curve.

Calculating the gaseous fluxes concentrations, from set time periods was achieved by the following equation (adapted from Levy et al. 2011):

$$\text{Flux } (\mu g\ m^{-2}\ h^{-1}) = \frac{\delta C}{\delta t} \times \left(\frac{V \times M}{a \times V_{mol}}\right)$$

Where δC is rate of change in the gas concentration over the time period; δt is the change in time from the background reading to the final measurement in hours; V is volume of the headspace of the chamber (m^3); M is the molecular weight of the gas; a is the area of the surface of the mesocsom, this is substituted for mass (g) of the soil/water sample in the microcosms and the units changed accordingly; V_{mol} is the volume of a mole of gas (air) at a given temperature ($m^3\ mol^{-1}$) calculated by:

$$p \times (R \times K)$$

Where p is pressure (kPa); R is equal to 8.314 (the ideal gas constant) and K is temperature (Kelvin). We used global warming carbon dioxide equivalents of 34 for CH_4 and 298 for N_2O (Myhre et al., 2013) on all measurements.

4.3.5 Statistical analysis

All statistical analyses were performed using Minitab v.16. The normality of data were tested for using the Kolmogorov-Smirnov test; data that proved not normally distributed were transformed using a Johnson's transformation (Johnson, 1995), which transforms the data to follow a normal distribution. We tested for statistical differences in microcosm enzyme activity and N_2O production between treatments separately for each time point using ANOVA with Tukey HSD *post hoc* tests.

Mesocosm enzyme activity and gas data were averaged for each treatment over the three day sampling period. Differences in the mesocosm enzyme activity, soil chemistry and greenhouse gas production were tested separately for each time point using general linear models ("water table" "nitrogen" "water table * nitrogen"). The model tested for the individual differences caused by the wet and dry treatments, nitrogen treatments and their interactions.

4.4 Results

4.4.1 Microcosm

In the microcosm experiment, the enzyme assays showed that POX activity was significantly higher ($F= 4.11$ $df= 3$ $p= 0024$) in the 10 mg l^{-1} DIN groundwater treatment than the 0 mg l^{-1} at 72 hours post treatment addition (Fig 4.2 c), yet was not significantly different at 24 hours. NAG and BG were also not significantly different between groundwater DIN treatments (at both 24 and 72 hours after groundwater nitrogen treatment addition, Fig 4.2 a & b). NAG and BG hydrolase enzyme activities within all treatments, however decreased from the 24 hour sampling point to the 72 hour sampling point (Fig 4.2 a & b). Soil moisture percentage decreased from 36.10 ± 0.45 at the 24 hour sampling point to 35.31 ± 0.33 at the 72 hour sampling point, although this data is not shown.

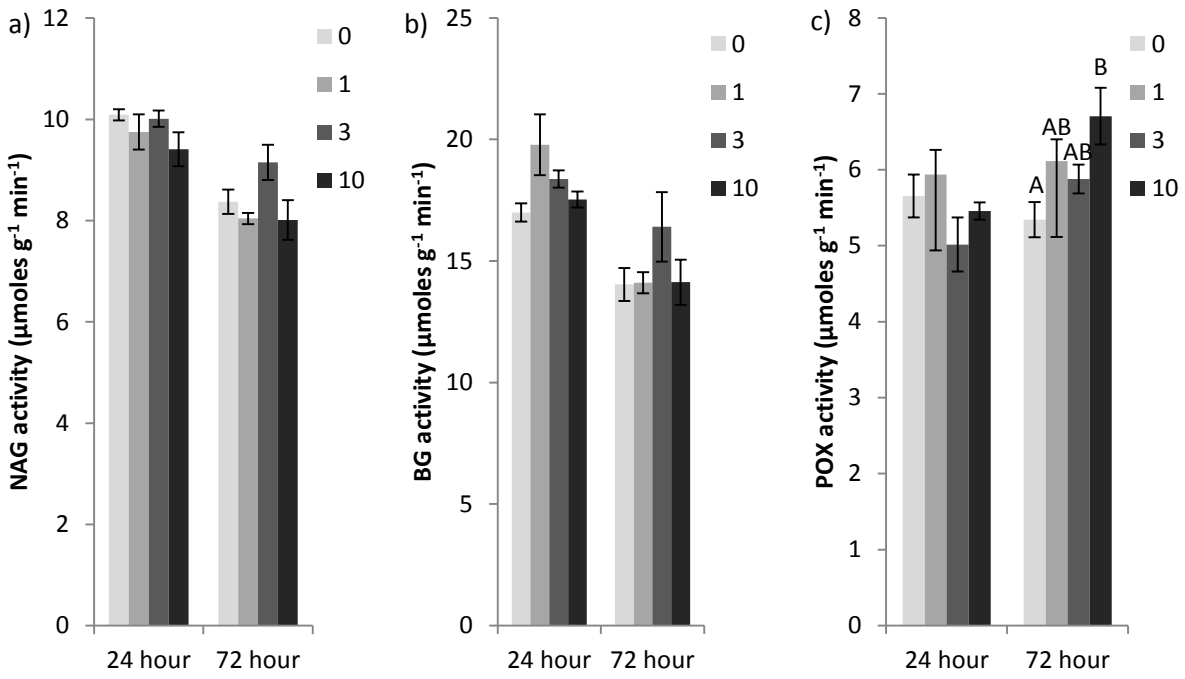


Fig 4.2 The activity of enzyme a) N-acetyl-β-glucosaminidase (NAG), b) β-glucosidase (BG) and c) Phenol oxidase (POX) in microcosm soils 24 hours and 72 hours after the addition of different groundwater DIN treatments (0, 1, 3 and 10 mg l^{-1} of DIN). Enzyme activity is reported as a mean ± one standard error. Letters denote significance between treatments at the 24 hour and 72 hour time points respectively.

N_2O gases were measured at intervals to help identify the time course of denitrification activity within the soils following the addition of DIN groundwater treatments. Negligible amounts of N_2O gas were produced within all DIN treatments for the first 8 hours post treatment addition (Fig 4.3). At 24 hours post DIN treatment addition a peak in N_2O production was measured for all treatment concentrations, with significantly higher production ($F= 3.86$ $df= 3$ $p= 0.025$) in the 10mg l^{-1} nitrogen treatment than the 3 mg l^{-1} treatment (Fig 4.3). At 48 hours N_2O gas production had decreased within all treatments (Fig 4.3), but remained elevated ($F= 2.37$ $df=3$ $p= 0.000$) in the 3 mg l^{-1} and 10 mg l^{-1} nitrogen treatments compared with the 0 mg l^{-1} and 1 mg $^{-1}$ nitrogen treatments, and was substantially higher in the 10 mg l^{-1} nitrogen treatment than in the 3 mg l^{-1}.

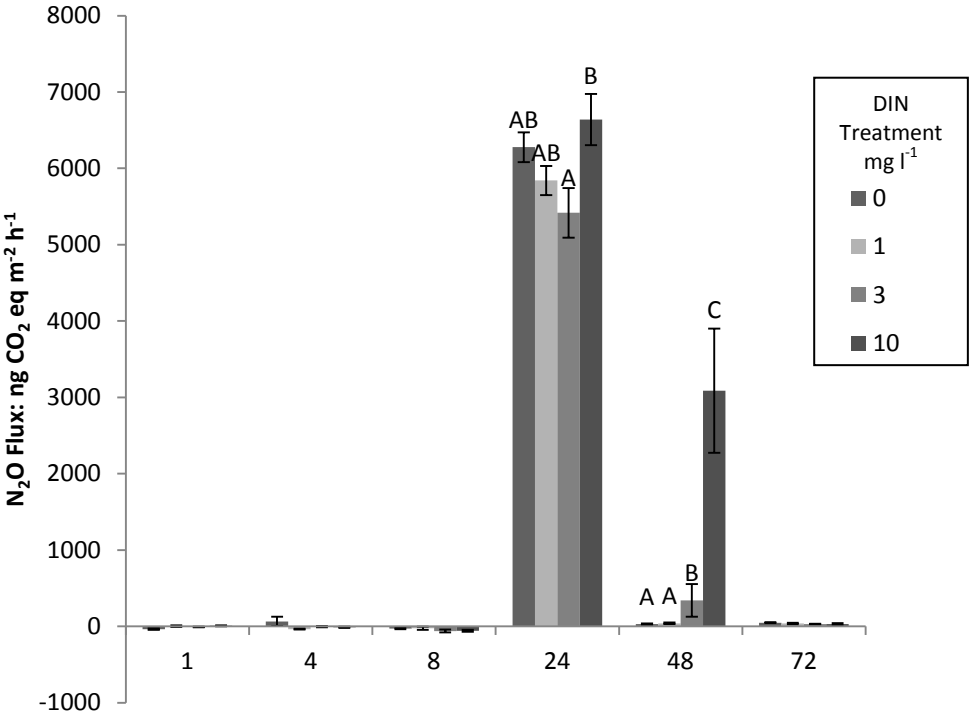


Fig 4.3 Time series of N_2O production following the addition of groundwater DIN treatments. N_2O production is reported as mean ± one standard error. Letters denote significance within individual time points.

4.4.2 Mesocosm

In the mesocosm experiment both groundwater DIN treatments and the interaction between both groundwater nitrogen treatments and water tables had no significant effect on groundwater DIN treatments soil chemical parameters, enzyme activity or greenhouse gas fluxes. The hydrological regimes (wet or dry treatment), within the mesocosm study, however, showed effects on soil chemical parameters, enzyme activity and greenhouse gas fluxes (see below), where soil moisture content at the end of July was 31.83 ± 0.37% within the wet treatment and 28.07 ± 0.47% in the dry. Soil nitrite ($F= 18.77$ $df= 1$ $p= 0.000$), nitrate ($F= 30.56$ $df= 1$ $p=0.000$) and DIN concentrations ($F= 22.72$ $df= 1$ $p= 0.000$) were significantly higher within the dry treatment than those exposed to the wet treatment (Fig 4.4 a,b & c), whilst TC and DIC concentrations were significantly lower within the dry treatments than within the wet treatments (Fig 4.4 d & e). Soil DOC: DON ratios (Fig 4.4 f) were unaffected by hydrological regimes.

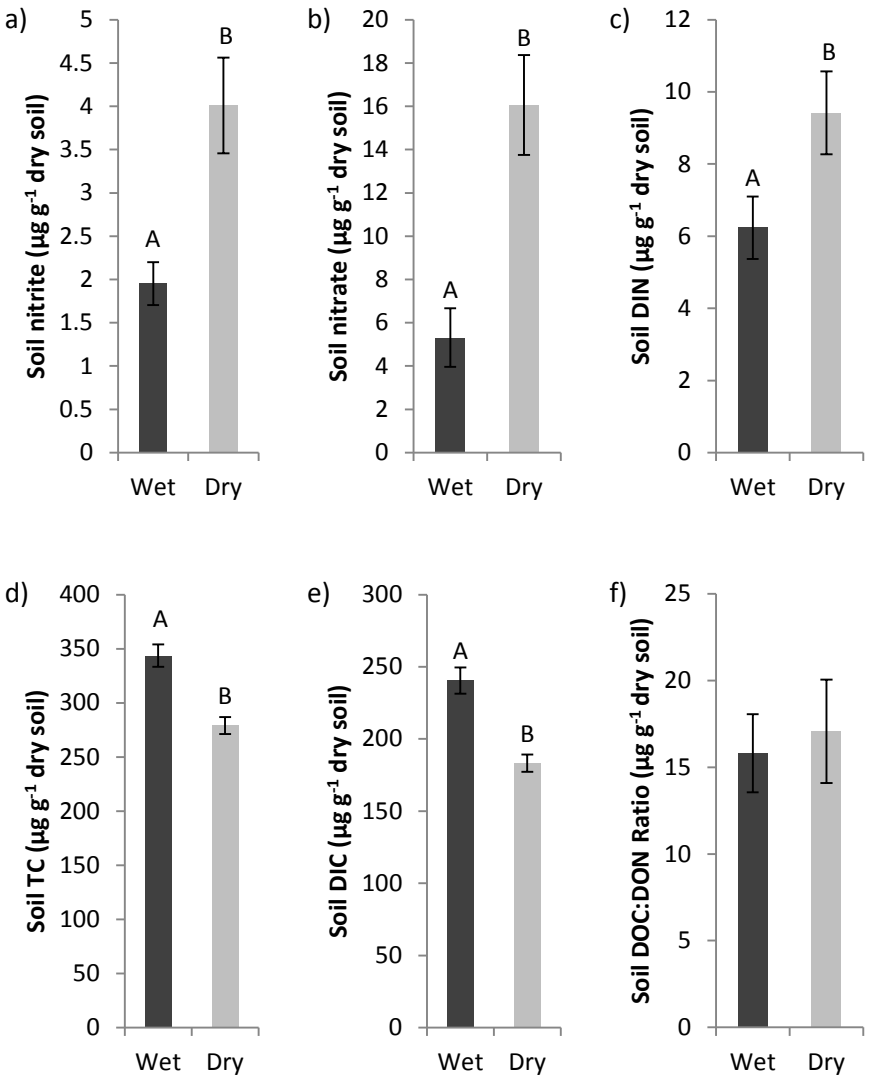


Fig 4.4 Mean water extractable soil a) nitrite b) nitrate c) DIN d) TC e) DIC and f) DOC: DON ratio. Different letters denote significance between wet and dry hydrological treatments.

Hydrolase enzyme activities NAG and BG were significantly affected by the hydrological treatment, where soil NAG activity was significantly higher (F= 4.32 df= 1 p=0.048) within the wet treatment than within the dry treatment (Fig 4.5 a) and BG activity was significantly lower (F= 4.48 df=1 p= 0.042) within the wet treatment than within the dry treatment (Fig 4.5 b). POX enzyme activity showed no significant differences between wet and dry treatments (Fig 4.5 c).

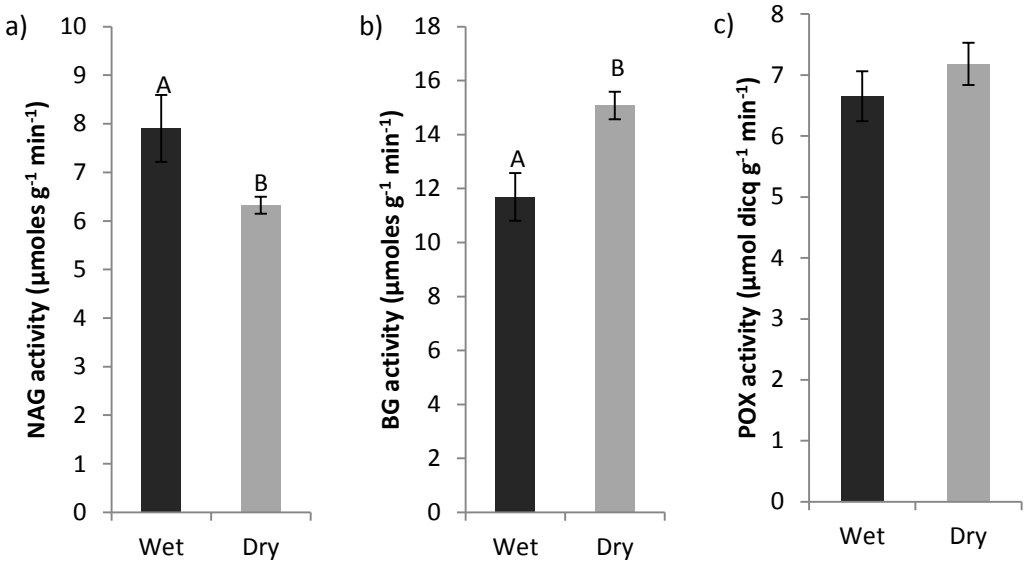


Fig 4.5 The activity of enzyme a) N-acetyl-β-glucosaminidase (NAG) b) β-glucosidase (BG) and c) Phenol oxidase (POX) within mesocosm soils. Different letters denote significance between wet and dry hydrological treatments.

Hydrological treatment had no effect on CO_2 uptake (i.e. through photosynthesis) measured on winter (29/01/14) and summer days (22nd to 24th of July), however CO_2 emissions measured on a summer night (21/07/14) were significantly higher ($F= 15.07$ $df= 1$ $p= 0.001$) within wet treatments compared to dry treatments (Fig 4.6 a). Methane emissions measured on a winter day ($F= 5.82$ $df= 1$ $p= 0.024$), summer day ($F= 39.84$ $df= 1$ $p= 0.000$) and summer night ($F= 38.80$ $df= 1$ $p=0.000$) were all significantly greater within mesocosms subject to the wet treatment than the dry treatment; with greater methane emissions within the summer than in the winter (Fig 4.6 b). Both the hydrological and DIN groundwater treatment had no significant effect on N_2O fluxes; however there was a consistent, yet non-significant trend for increasing positive N_2O fluxes with increasing groundwater nitrogen treatment (Fig 4.6 c). Even though groundwater DIN treatment had no effect on greenhouse gas fluxes, there was a consistent, but non-significant, trend of increased N_2O emissions with increasing groundwater nitrogen treatment (Fig 4.7).

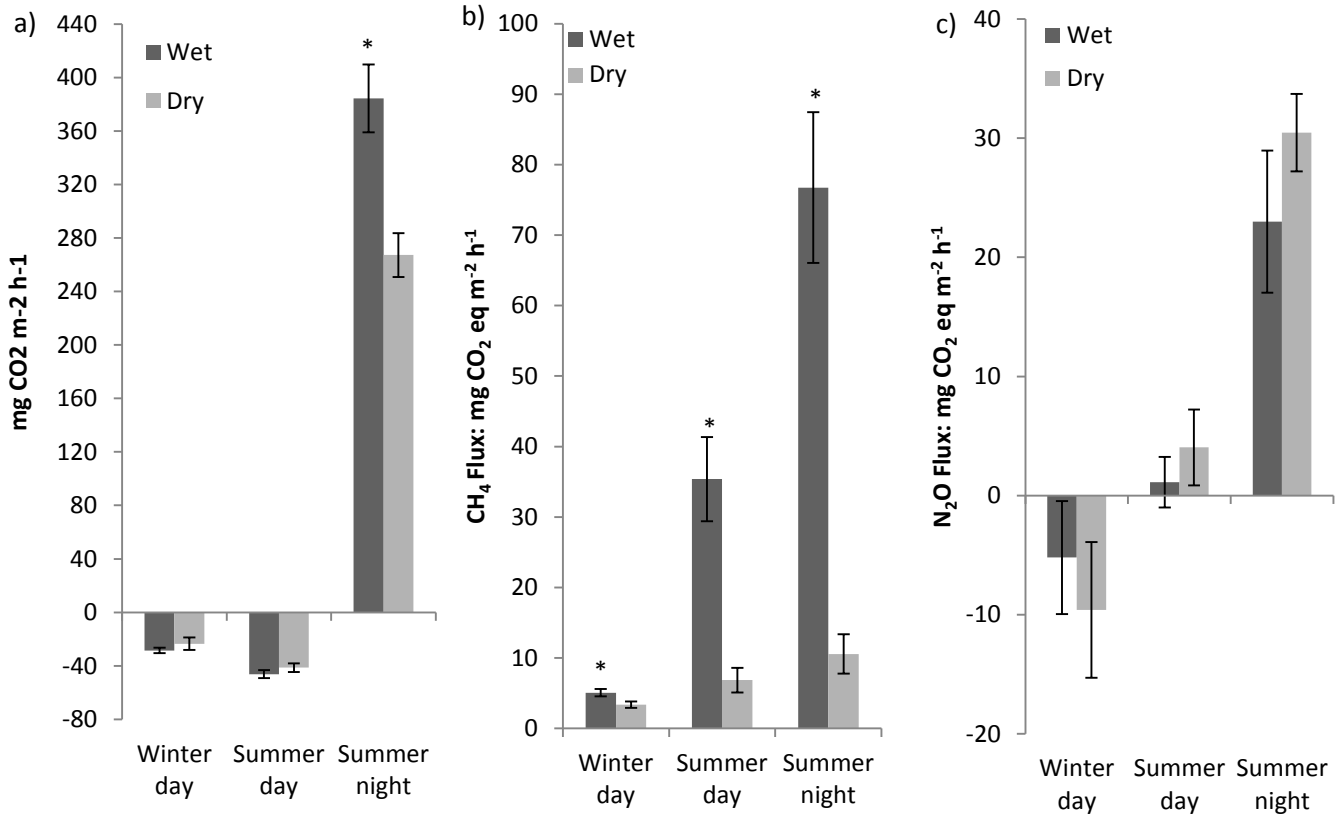


Fig 4.6 Wet and dry hydrological treatment effects on the emissions of a) $CO_2$and b) CH_4 and c) N_2O. Gas samples collected on a winters day (29/01/14), summer day (coincides with soil sampling for enzyme activity, 22nd to 24th of July) and summers night (21/07/14). Comparisons were only made between treatments for a winter day, summer day and summer night. An asterisk denotes significance between wet and dry hydrological treatments at a single time point (i.e. winter day).

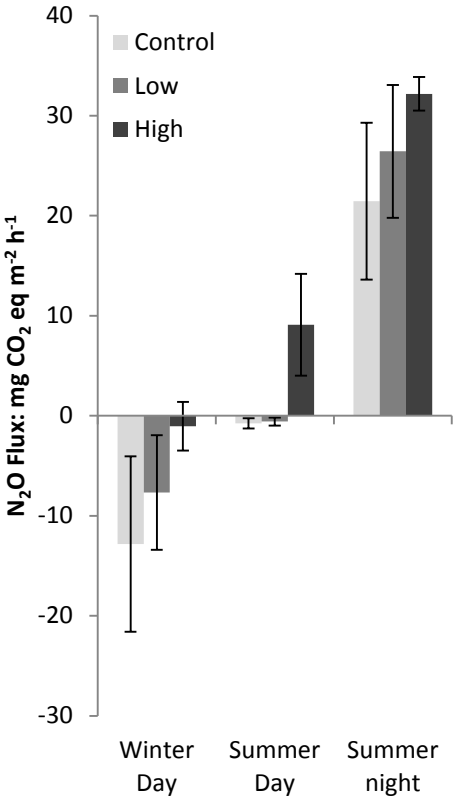


Fig 4.7 The effects of groundwater nitrogen treatment (Control, low and high) on N_2O emissions. Gas samples collected on a winters day (29/01/14), summer day (coincides with soil sampling for enzyme activity, 22nd to 24th of July) and summers night (21/07/14). Comparisons were only made between treatments for a winter day, summer day and summer night.

4.5 Discussion

This study investigated the potential impacts of eutrophication and climate change on dune slack soil processes and, specifically, the potential effects of groundwater DIN and soil moisture content on denitrification rates, carbon cycling and greenhouse gas emissions.

Previous studies demonstrate the temporal variability of soil denitrification in response to increases in soil water content (Rudaz et al., 1991, Martin et al., 1988), with N_2O production measured within 30 minutes in dry grassland soils (Rudaz et al., 1991) and longer in other studies. In the microcosms, due to the nature of the experiment, soils experienced an immediate increase in soil water content following treatment addition, rather than a gradual increase in soil water content, from a slow water table increase. Nevertheless, this is consistent with a heavy rainfall event or a sudden increase in water table depth following sustained rainfall. We found that under these conditions denitrification lasted longer within dune slack soils with higher groundwater nitrogen concentrations. As

denitrification has been found to significantly increase with N availability (Merrill and Zak, 1992), it is likely that N stores within the higher DIN treatments are larger than those treated with lower DIN treatments, causing denitrification activity rates to last longer.

Higher water levels increase the thickness of the anaerobic zone within a soil profile and decrease the thickness of the aerobic zone. Increased NAG activities within the mesocosm wet treatment were observed (Fig 4.4 a), consistent with more favourable anaerobic conditions for denitrification. This was probably the cause of the lower nitrite, nitrate and DIN concentrations within the wetter soils (Fig 4.3 a, b & c). In the same mesocosm study however, no significance between DIN groundwater treatments in soil nitrogen chemistry and NAG activity were found, yet there was a clear increasing N_2O production trend with increasing nitrogen groundwater treatments. This was also consistent with the significant results in the microcosm study, where microcosms subject to higher groundwater nitrogen concentrations were seen to produce N_2O for a longer period of time following nitrogen treatment addition; although no effect on NAG activity was observed. Therefore it is likely that groundwater nitrogen was increasing denitrification and therefore N_2O emission, and this could be due to the uptake of nitrogen by other enzymes such as L-leucine aminopeptidase and urease (Sinsabaugh et al., 2002), which breakdown different classes of substrates to NAG.

The effect of nitrogen addition on carbon cycling, and POX activity in particular, varies significantly across different studies and the responses are largely explained by the quantity of lignin in plant litter (Waldrop et al., 2004). Peatland soils subject to increased atmospheric nitrogen, with characteristically low amounts of lignin are seen to have higher POX activity (Bragazza et al., 2006). As dune slack soils are subject to low amounts of litter fall, they have lower amounts of lignin, which is similar to peatland soils. Our findings are in accordance with those of Bragazza et al. (2006), where POX activity increased within microcosms subject to higher groundwater nitrogen contamination 72 hours after nitrogen treatment addition. In turn, the increase in POX activity reduces polyphenol concentrations and indirectly stimulates the activity of hydrolase enzymes, such as NAG and BG, by means of the phenol oxidase latch mechanism (Freeman et al., 2001), which ultimately increases decomposition rates. The latch response however was not observed within this study, with unaffected NAG and BG activity from increased POX activity. Nonetheless, the increase in POX activity suggests that dune slack soil decomposition rates are sensitive to groundwater nitrogen contamination leading to increased decomposition and subsequently the potential for reduced carbon sequestration.

On the other hand, the mesocosm study also incorporated the impacts from lowered water tables, where we found no significant impact from groundwater nitrogen contamination on dune slack carbon related soil processes. A 10 cm lowering in water table however was seen to primarily affect soil chemistry, enzyme activity and greenhouse gas emissions. Within anaerobic soils where soil temperatures are low and microbial biomasses are small, soil respiration and decomposition rates are reduced, which conserves carbon within the soil for longer periods (Kang and Freeman, 1999, Flanagan and Syed, 2011).

Here, we observed higher concentrations of TC within the wet treatment because of increased contributions of DIC (dissolved CO_2, See explanation below), rather than DOC. and, since BG activity decreased (Fig 4 b) and anaerobic conditions increased, which are known to favour incomplete decomposition and methanogenisis, this could be explained by the higher night CO_2 fluxes within the wet treatment, including both plant and microbial respiration. The higher CO_2 fluxes may be a result of increased root respiration and/or the incomplete decomposition of soil organic matter under such anoxic conditions (Whalen, 2005), or increased methanogenesis, where CO_2 can also result as a by-product of methane production, dependant on the nature of the terminal electron acceptor or by anaerobic methane oxidation (Ferry, 1993); indeed, here, methane production was significantly higher within wetter dune slack soils, in line with the consensus (Segers, 1998, Whalen, 2005). These findings are consistent with the moisture content within the wet and dry treatment. Limited data also suggests that uptake of CO_2 within the winter and summer months indicate that CO_2 intake from vegetation photosynthesis is greater than CO_2 emissions from soil respiration.

In broad agreement with the literature (e.g. Whalen, 2005), methane production was greater within wetter dune slack soils in all seasons measured, which was expected due to methane production primarily being an anaerobic process (Segers, 1998, Whalen, 2005). The lower water table within the dry treatment increased the thickness of the oxic zone within the soil profile, which would encourage methanotrophic bacterial processes which consume methane and therefore limit the amount of methane released to the atmosphere (Pearce and Clymo, 2001). As methane emissions are sensitive to soil conditions and temperature (Whalen, 2005), methane emissions show temporal and seasonal variation.

BG activity was significantly higher within the dry treatment, suggesting increased degradation of cellulose within these soil conditions, which could be as a result of an increase in microbial biomass (Turner et al., 2002) resulting in increased enzyme synthesis. This is unlikely however as if this was the case we would see similar results with POX enzyme activity. This suggests that that the BG

enzyme is being synthesised by soil microorganisms in response to the occurrence of appropriate carbon substrates or by the increase of inorganic nutrients (Fenner and Freeman, 2011), in this case, soil nitrate and nitrite. Phenol oxidase was not significantly affected by water table, which could be due to the extremely low availability of substrate for this enzyme, i.e. phenolic concentrations (Freeman et al., 1996), found within sandy soils. However, this was not measured here. These findings therefore suggest that with lowered water tables, cellulose decomposition rates are likely to increase, through BG activity, without demonstrable increases of soil respiration. However, from the microcosm study the intensified phenolic decomposition rates found under groundwater nitrogen contamination indicates likely carbon losses.

4.6 Conclusions

Our findings suggest that dune slack habitats are highly sensitive to both groundwater nitrogen addition and modest changes in water tables. Should drier conditions prevail, as a result of climate change (Clarke and Ayutthaya, 2010) or water abstraction, dune slack soils are likely to become drier and, in turn, this will reduce denitrification rates, leading to greater nitrogen retention and therefore a greater eutrophication impact. Nevertheless, the global availability of nitrogen is increasing (Galloway and Cowling, 2002) and it is therefore likely that DIN availability within dune slack soils will still increase, irrespective of water table changes. Subsequently, dune slacks are likely to experience plant community shifts from both a decrease in soil water content (Curreli et al., 2013) and an increase in soil nitrogen availability (Rhymes et al., 2014), both posing a serious threat to endangered dune slack species.

With regard to carbon cycling within dune slack soils, BG measurements suggest that decomposition rates are increased with lowered water tables, even though there were no demonstrable increases in soil respiration, whilst the impacts from groundwater nitrogen contamination are also likely to increase decomposition rates.

Overall, this study indicates that the chemical composition and soil processes within dune slack soils are sensitive to both groundwater nitrogen availability, minimal alterations in water tables and the interaction of both. The predicted lowering of water tables (Curreli et al., 2013) and potential increase in global nitrogen availability are likely to alter dune slack soils, with soils becoming eutrophic and drier, subsequently altering dune slack vegetation assemblages (Curreli et al., 2013, Rhymes et al., 2014). These threats will also increase soil decomposition rates, and thereby potentially reducing carbon sequestration in this habitat. Taken together, our findings suggest that there is a hierarchy with hydrology being the dominant factor followed by nitrogen contamination,

and so climate change poses more of a threat than eutrophication. The response of dune slack soil processes to such threats are still poorly understood and further research is required to understand the future prospects of dune slack ecology and wetlands in general in a changing environment.

4.7 References

BALL, D. F. 1963. The soils and land use of the district around Bangor and Beaumaris [sheets 94 and 106]. *The soils and land use of the district around Bangor and Beaumaris [sheets 94 and 106].*

BERENDSE, F., LAMMERTS, E. & OLFF, H. 1998. Soil organic matter accumulation and its implications for nitrogen mineralization and plant species composition during succession in coastal dune slacks. *Plant Ecology,* 137**,** 71-78.

BERNOT, M. J., DODDS, W. K., GARDNER, W. S., MCCARTHY, M. J., SOBOLEV, D. & TANK, J. L. 2003. Comparing denitrification estimates for a Texas estuary by using acetylene inhibition and membrane inlet mass spectrometry. *Applied and Environmental Microbiology,* 69**,** 5950-5956.

BRADY, N. C. & WEIL, R. R. 2002. The nature and property of soils. Upper Saddle River, NJ: Prentice Hall.

BRAGAZZA, L., FREEMAN, C., JONES, T., RYDIN, H., LIMPENS, J., FENNER, N., ELLIS, T., GERDOL, R., HÁJEK, M. & HÁJEK, T. 2006. Atmospheric nitrogen deposition promotes carbon loss from peat bogs. *Proceedings of the National Academy of Sciences,* 103**,** 19386-19389.

BURGIN, A. J., GROFFMAN, P. M. & LEWIS, D. N. 2010. Factors regulating denitrification in a riparian wetland. *Soil Science Society of America Journal,* 74**,** 1826-1833.

CAMARGO, J. A. & ALONSO, A. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International,* 32**,** 831-849.

CHANTIGNY, M. H. 2003. Dissolved and water-extractable organic matter in soils: a review on the influence of land use and management practices. *Geoderma,* 113**,** 357-380.

CLARKE, D. & AYUTTHAYA, S. S. N. 2010. Predicted effects of climate change, vegetation and tree cover on dune slack habitats at Ainsdale on the Sefton Coast, UK. *Journal of Coastal Conservation,* 14**,** 115-125.

CURRELI, A., WALLACE, H., FREEMAN, C., HOLLINGHAM, M., STRATFORD, C., JOHNSON, H. & JONES, L. 2013. Eco-hydrological requirements of dune slack vegetation and the implications of climate change. *Science of the Total Environment,* 443**,** 910-919.

DELAUNE, R. & JUGSUJINDA, A. 2003. Denitrification potential in a Louisiana wetland receiving diverted Mississippi River water. *Chemistry and Ecology,* 19**,** 411-418.

DENG, S., & POPOVA, I. 2011. Carbohydrate hydrolases. In: Dick RP (ed) Mehods of soil enzymology. *Soil Science Society of America, Inc., Madison,*pp 185-209.

DUNN, C., JONES, T. G., GIRARD, A. & FREEMAN, C. 2014. Methodologies for Extracellular Enzyme Assays from Wetland Soils. *Wetlands,* 34**,** 9-17.

FENNER, N. & FREEMAN, C. 2011. Drought-induced carbon loss in peatlands. *Nature Geoscience,* 4**,** 895-900.

FERRY, J. G. 1993. *Methanogenesis: ecology, physiology, biochemistry & genetics*, Springer.

FLANAGAN, L. B. & SYED, K. H. 2011. Stimulation of both photosynthesis and respiration in response to warmer and drier conditions in a boreal peatland ecosystem. *Global Change Biology,* 17**,** 2271-2287.

FREEMAN, C., LISKA, G., OSTLE, N., LOCK, M., REYNOLDS, B. & HUDSON, J. 1996. Microbial activity and enzymic decomposition processes following peatland water table drawdown. *Plant and Soil,* 180**,** 121-127.

FREEMAN, C., LISKA, G., OSTLE, N. J., JONES, S. E. & LOCK, M. A. 1995. The Use of Fluorogenic Substrates for Measuring Enzyme-Activity in Peatlands. *Plant and Soil,* 175**,** 147-152.

FREEMAN, C., OSTLE, N. & KANG, H. 2001. An enzymic 'latch' on a global carbon store - A shortage of oxygen locks up carbon in peatlands by restraining a single enzyme. *Nature,* 409**,** 149-149.

GALLOWAY, J. N. & COWLING, E. B. 2002. Reactive nitrogen and the world: 200 years of change. *Ambio,* 31**,** 64-71.

GROFFMAN, P. M., ALTABET, M. A., BÖHLKE, J., BUTTERBACH-BAHL, K., DAVID, M. B., FIRESTONE, M. K., GIBLIN, A. E., KANA, T. M., NIELSEN, L. P. & VOYTEK, M. A. 2006. Methods for measuring denitrification: diverse approaches to a difficult problem. *Ecological Applications,* 16**,** 2091-2122.

GROOTJANS, A., ADEMA, E., BEKKER, R. & LAMMERTS, E. 2004. Why coastal dune slacks sustain a high biodiversity. *Coastal Dunes.* Springer.

HEFTING, M., CLEMENT, J., DOWRICK, D., COSANDEY, A., BERNAL, S., CIMPIAN, C., TATUR, A., BURT, T. & PINAY, G. 2004. Water table elevation controls on soil nitrogen cycling in riparian wetlands along a European climatic gradient. *Biogeochemistry,* 67**,** 113-134.

HENRY, H. A. L. 2012. Soil extracellular enzyme dynamics in a changing climate. *Soil Biology and Biochemistry,* 47**,** 53-59.

JOHNSON, D. H. 1995. Statistical sirens: the allure of nonparametrics. *Ecology*, 1998-2000.

JONES, L., NIZAM, M., REYNOLDS, B., BAREHAM, S. & OXLEY, E. 2013. Upwind impacts of ammonia from an intensive poultry unit. *Environmental Pollution,* 180**,** 221-228.

JONES, M. L. M., SOWERBY, A., WILLIAMS, D. L. & JONES, R. E. 2008. Factors controlling soil development in sand dunes: evidence from a coastal dune soil chronosequence. *Plant and Soil,* 307**,** 219-234.

KANG, H. & FREEMAN, C. 1999. Phosphatase and arylsulphatase activities in wetland soils: annual variation and controlling factors. *Soil Biology and Biochemistry,* 31**,** 449-454.

KANG, H., FREEMAN, C. & CHUN, J. 2005. N-Acetylglucosaminidase activities in wetlands: a global survey. *Hydrobiologia,* 532**,** 103-110.

KNOWLES, R. 1982. Denitrification. *Microbiological Reviews,* 46**,** 43-70.

LEVY, P., GRAY, A., LEESON, S., GAIAWYN, J., KELLY, M., COOPER, M., DINSMORE, K., JONES, S. & SHEPPARD, L. 2011. Quantification of uncertainty in trace gas fluxes measured by the static chamber method. *European Journal of Soil Science,* 62**,** 811-821.

MARTIN, K., PARSONS, L. L., MURRAY, R. E. & SMITH, M. S. 1988. Dynamics of soil denitrifier populations: relationships between enzyme activity, most-probable-number counts, and actual N gas loss. *Applied and Environmental Microbiology,* 54**,** 2711-2716.

MCLATCHEY, G. P. & REDDY, K. 1998. Regulation of organic matter decomposition and nutrient release in a wetland soil. *Journal of Environmental Quality,* 27**,** 1268-1274.

MERRILL, A. G. & ZAK, D. R. 1992. Factors Controlling Denitrification Rates in Upland and Swamp Forests. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere,* 22**,** 1597-1604.

MYHRE, G. D., SHINDELL, D., BREON, F. M., COLLINS, W., FUGLESTVEDT, J., HUANG, J., KOCH, D., LAMARQUE, J. F., LEE, D., MENDOZA, B., NAKAJIMA, T., ROBOCK, A., STEPHANS, G., TAKEEMURA, T., and ZHANG, H.: Anthropogenic and Natural Radiative Forcing, in: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, Chap. 8, 2013.PEARCE, D. M. E. & CLYMO, R. S. 2001. Methane oxidation in a peatland core. *Global Biogeochemical Cycles,* 15**,** 709-720.

PIND, A., FREEMAN, C. & LOCK, M. A. 1994. Enzymatic Degradation of Phenolic Materials in Peatlands - Measurement of Phenol Oxidase Activity. *Plant and Soil,* 159**,** 227-231.

PLASSMANN, K., EDWARDS-JONES, G. & JONES, M. L. M. 2009. The effects of low levels of nitrogen deposition and grazing on dune grassland. *Science of the Total Environment,* 407**,** 1391-1404.

PLASSMANN, K., JONES, M. L. M. & EDWARDS-JONES, G. 2010. Effects of long-term grazing management on sand dune vegetation of high conservation interest. *Applied Vegetation Science,* 13**,** 100-112.

REMKE, E., BROUWER, E., KOOIJMAN, A., BLINDOW, I., ESSELINK, H. & ROELOFS, J. G. 2009. Even low to medium nitrogen deposition impacts vegetation of dry, coastal dunes around the Baltic Sea. *Environmental Pollution,* 157**,** 792-800.

RHYMES, J., WALLACE, H., FENNER, N. & JONES, L. 2014. Evidence for sensitivity of dune wetlands to groundwater nutrients. *Science of the Total Environment,* 490C**,** 106-113.

ROBINS, N. & JONES, M. 2013. Ecohydrological 'indicators of alteration'–a robust measure of change in dune slacks. *Ecohydrology,* 6**,** 256-263.

RUDAZ, A. O., DAVIDSON, E. A. & FIRESTONE, M. K. 1991. Sources of nitrous oxide production following wetting of dry soil. *FEMS Microbiology Letters,* 85**,** 117-124.

SEGERS, R. 1998. Methane production and methane consumption: a review of processes underlying wetland methane fluxes. *Biogeochemistry,* 41**,** 23-51.

SEITZINGER, S., HARRISON, J. A., BÖHLKE, J., BOUWMAN, A., LOWRANCE, R., PETERSON, B., TOBIAS, C. & DRECHT, G. V. 2006. Denitrification across landscapes and waterscapes: a synthesis. *Ecological Applications,* 16**,** 2064-2090.

SINSABAUGH, R., CARREIRO, M. & REPERT, D. 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. *Biogeochemistry,* 60**,** 1-24.

STRATFORD, C. J., ROBINS, N. S., CLARKE, D., JONES, L. & WEAVER, G. 2013. An ecohydrological review of dune slacks on the west coast of England and Wales. *Ecohydrology,* 6**,** 162-171.

TURNER, B. L., HOPKINS, D. W., HAYGARTH, P. M. & OSTLE, N. 2002. β-Glucosidase activity in pasture soils. *Applied Soil Ecology,* 20**,** 157-162.

VAN DER LAAN, D. 1979. Spatial and temporal variation in the vegetation of dune slacks in relation to the ground water regime. *Vegetatio,* 39**,** 43-51.

WALDROP, M. P., ZAK, D. R., SINSABAUGH, R. L., GALLO, M. & LAUBER, C. 2004. Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. *Ecological Applications,* 14**,** 1172-1177.

WHALEN, S. 2005. Biogeochemistry of methane exchange between natural wetlands and the atmosphere. *Environmental Engineering Science,* 22**,** 73-94.

WILLIS, A., FOLKES, B., HOPE-SIMPSON, J. & YEMM, E. 1959. Braunton Burrows: the dune system and its vegetation. *The Journal of Ecology***,** 249-288.

CHAPTER 5: **The effects of groundwater nitrogen contamination and climate change on dune slack ecology**

J. Rhymes [1], L. Jones [2], H. Wallace [3], S.Y. Tang [2], T. Jones [1], N. Fenner [1]

[1]Bangor University, UK; [2]Centre for ecology and hydrology, UK; [3] Ecological surveys (Bangor), UK

Author Contributions: The chapter was written by Jennifer Rhymes. Laurence Jones, Nathalie Fenner and Hilary Wallace provided advice on the experimental design and gave editorial suggestions. Christian Dunn and Timothy Jones provided technical laboratory advice. Sing Tang analysed gaseous ammonia ALPHA samplers.

5.1 Abstract

Dune slacks are seasonal wetlands which experience considerable fluctuations in water table depths. They are of biological importance because they are high in biodiversity. Dune slacks are subject to multiple threats such as climate change and eutrophication. The biological effects caused by the interactions of both of these pressures are poorly understood. In this study we measure the impact of groundwater nitrogen contamination, lowered water table depth and the interactions of these factors on evapotranspiration rates, the growth of individual species and plant tissue chemistry. We also quantified the uptake and losses of nitrogen within these systems. Results indicate that nitrogen uptake and losses within dune slack habitats is increased with increased groundwater dissolved inorganic nitrogen (DIN) concentrations, with evidence of nutrient uptake by vegetation. It is proposed that fluxes of uptake and losses within a natural sand dune system are high, highlighting the likelihood of nitrogen loads being underestimated within these habitats. Furthermore a 10 cm drop in water table depth, simulating climate change, showed higher water losses in the wetter dune slack mesocosms. Lowered water tables also affected percentage plant cover in a forb and sedge species.

5.2 Introduction

Wet dune slacks are seasonal wetlands that occur within the low-lying areas within a sand dune system. In Europe they support a diverse flora (Grootjans et al., 2004), which are sensitive to multiple changes, such as eutrophication and lowered water tables (Provoost et al., 2011), from either climate change (Clarke and Ayutthaya, 2010) or water abstraction. The predicted drop in water table depths as a result of climate change (Clarke and Ayutthaya, 2010) and the increasing availability of nitrogen from anthropogenic modifications (Galloway and Cowling, 2002) poses a serious threat to these dune slack habitats.

Dune slacks experience seasonal variations in hydrological regimes, where water table depths are at their highest in the winter and lowest in the summer (van der Laan, 1979). Water level is highly dependent on precipitation and evapotranspiration rates, although the latter is also dependent on water table depth (Stratford et al., 2013). Dune slack species distribution and community structure are primarily influenced by groundwater levels (Willis et al., 1959, van der Laan, 1979, Curreli et al., 2013) and the differences in wet and dry dune slack species composition is well described (e.g. Rodwell et al., 2000). Recently, Curreli et al. (2013) found that a difference in water table depth of 40 cm separates the wettest dune slack communities from the driest. Dune slack communities can be influenced by groundwater nitrogen contamination independently of water table depth, whereby nitrophilous species out-compete endangered basipholous dune slack species (Rhymes et al., 2014). Dune slack hydrological fluctuations however play a key role in conserving the low nutrient status required by dune slack species (Berendse et al., 1998). Furthermore, the predicted decline in water table depth of 100 cm by 2080 (Clarke and Ayutthaya, 2010) is likely to reduce denitrification rates (Rhymes et al., 2015, in prep.) with potential for ecological impacts.

Previous studies that have investigated the impacts of nitrogen contamination on dune slack ecology have primarily concentrated on atmospheric inputs. Increased atmospheric deposition has been seen to increase tissue nitrogen content within dune species (Jones et al., 2013, Plassmann et al., 2009), increase above ground biomass (Plassmann et al., 2009) and alter species composition (Remke et al., 2009). Nutrients from atmospheric deposition have increased significantly from their pre-industrial levels of 2-6 kg N ha^{-1} yr^{-} (Fowler et al., 2005), resulting in dune systems across much of Europe now exceeding the current critical load defined for dune slacks of 10-15 kg N ha^{-1} yr^{-} (Bobbink and Hettelingh, 2010). In addition, the likely increase in groundwater nitrogen availability from increased agricultural practices (Galloway and Cowling, 2002) poses an additional threat to dune slack ecology from nitrogen contamination.

Groundwater nitrogen can enter sand dune sites that are not hydrologically isolated from surrounding contaminated groundwaters. The described nitrogen critical loads currently do not consider nitrogen derived from groundwater inputs (Bobbink and Hettelingh, 2010) and little attention has been given to the ecological impacts caused by groundwater contamination on dune slack ecology. A global assessment of aquatic ecosystems concluded that concentrations above 0.5-1.0 mg l^{-1} of total nitrogen (TN) could lead to eutrophication (Camargo and Alonso, 2006). A collation of dune groundwater chemistry specifically suggested that 1 mg l^{-1} of groundwater DIN indicates probable dune groundwater contamination (Davy et al., 2010). Recently, however, Rhymes et al. (2014) found that groundwater nitrogen contamination as low as 0.2 mg l^{-1} may have an ecological impact on both dune slack soil biogeochemistry and plant community structure. Nevertheless, the study did not quantify nitrogen loads, uptake or losses within the sand dune system and consequently there is no study to date that investigates and quantifies the fate of nitrogen within these systems, including the timescales within which nitrogen causes ecological impact.

The aim of this study was to investigate the interactions of groundwater nitrogen contamination and lowered water tables on the growth of individual species, dune slack plant tissue chemistry and the fate of nitrogen within these systems. We tested the following hypotheses: 1) Lowered water tables will decrease evapotranspiration rates, 2) The interaction of lowered water tables and groundwater nitrogen contamination will alter the growth of individual species and 3) Higher groundwater nitrogen contamination concentrations will increase the quantity of nitrogen taken up, lost and processed within dune slack ecosystems.

5.3 Materials and methods

5.3.1 Experimental material collection

Dune slack soil was collected from an uncontaminated *Salix repens-Calliergon cuspidatum stellatum* community dune slack at Aberffraw (Anglesey, North Wales, UK, 53°11'N, 4°27'W) and separated into two soil types: an organic top 10 cm layer and mineral sand from depth range -10 to -50 cm. Roots were removed by hand and homogenised with a cement mixer.

5.3.2 Mesocosm construction

Each mesocosm was constructed with plastic pipe (50 cm height and 16 cm diameter) with a mesh-lined perforated plastic base attached to the bottom for drainage. The first 42 cm was filled with mineral sand with no organic matter (described above), whilst the top 8 cm was filled with homogenised organic matter. Each mesocosm was planted with four typical dune slack species (2

sedges and 2 forb species); one specimen each of *Carex arenaria*, *Carex flacca*, *Leontodon autumnalis* and *Prunella vulgaris*. The mesocosms were then placed into individual buckets filled with artificial groundwater and treatments (see details below). Holes within the side of the buckets were used to control water table regimes and were attached to plastic tubing to collect any overflow. Black plastic was used to cover the opening of the bucket to exclude light, which did not permit rainfall to mix directly into the groundwater and to avoid water loss through evaporation. The outer part of the mesocosms, buckets and outlet bottles were wrapped in foil to minimise absorption of the sun's heat.

5.3.3 Experimental design

This experiment ran from October 2013 to July 2014 in Bangor, North Wales, UK (53°13'32.0"N, 4°07'55.1"W) and involved six DIN groundwater treatments; control (0.0 mg l^{-1} of DIN), low (0.2 mg l^{-1} of DIN) and high (10 mg l^{-1} of DIN) in factorial combination with a wet or dry hydrological regime, each with eight replicates. Wet hydrological regimes were altered from -10 cm water table depth in the winter months to -30 cm in the summer months, whilst the dry hydrological treatments were altered to consistently be 10 cm lower than the wet treatment. The artificial groundwater was synthesised by adding the listed compounds (Table 5.1) to 20L of de-ionised water, mimicking concentrations of cations, anions and the low pH measured at Aberffraw previously (Rhymes et al., 2014). Due to rainfall and evaporation water tables fluctuated in line with typical hydrological regimes in the field, although were unable to flood. On the 1st of July 2014 two litres of artificial groundwater (Table 5.1) was added to each mesocosm to avoid causing drought conditions within the mesocosms due to a long period without rainfall. Nitrogen treatments were maintained monthly by calculating amounts of ammonium nitrate required to meet targeted DIN treatment concentrations.

Table 5.1 Artificial groundwater recipe; compound weights added to 20L de-ionised water

Compound	**Weight (g)**
$CaCO_3$	0.941
$CaCl_2.6H_2O$	7.541
$MgSO_4.7H_2O$	0.370
$MgCl_2.6H_2O$	0.996
KCl	0.089
$NaHCO_3$	6.082

5.3.4 Water table depth, water chemistry sampling and maintenance of treatments

Manual water level depths from ground surface to water table were measured once a week. Volume of water within each mesocosm was estimated by the calculated volume of water from the water table depth within the bucket and the water held within the mesocosm sand based on a water-holding capacity of 30% (Ranwell, 1959). The groundwater chemical composition was measured on a monthly basis by taking a water sample from each bucket and filtering through 0.45 µm nylon syringe filter (Avonchem™). NO_3 and NH_4 concentrations were quantified by ion chromatography (Metrohm, UK Ltd.). DIN concentrations were calculated by the addition of NO_3-N and NH_4-N. Calculated DIN concentrations and bucket water volume were then used to calculate the volume of ammonium nitrate required for DIN concentrations to return to the target DIN treatment on a monthly basis

5.3.5 Nitrogen and water budget experiment

From May to July a water and nitrogen budget was calculated for each individual mesocosm. Inputs of water were rainfall and added groundwater stock. Rainfall was measured weekly. Measured outputs of water were water collected in the overflow bottles, measured bi-monthly. Budget calculations were made to calculate water losses from evapotranspiration on a monthly basis. These were then combined to give evapotranspiration losses over 3 months.

Nitrogen inputs measured included monthly DIN inputs from the ammonium nitrate treatments added (see above) and monthly atmospheric deposition concentrations and fluxes (described below). DIN concentrations from the overflow bottles were measured bi-weekly. Budget calculations were made to calculate losses of N via plant and soil uptake and denitrification on a monthly basis, which were then combined to give total N losses over a 3 month period. Temperature was also recorded in triplicate hourly (TinyTag Plus 2, accuracy ± 0.4 °C, Gemini data loggers, UK) and averaged for daily temperature values throughout the duration of this experiment.

Atmospheric nitrogen deposition measurements

A monitoring station located 3 meters away from mesocosms measured gaseous nitrogen dioxide and gaseous ammonia concentrations to calculate dry N deposition. Rainfall ammonium and nitrate concentrations and rainfall volume were measured to calculate wet N deposition during the three months. Gaseous nitrogen dioxide concentrations were sampled in triplicate using diffusion tubes supplied and analysed by Gradko International Ltd, Winchester, UK. Gaseous ammonia concentrations were measured using triplicate ALPHA samplers supplied and analysed by Centre for

Ecology and Hydrology, Edinburgh with a laboratory blank. Both sets of gaseous samplers were exposed monthly for a three-month period from May to July. Wet deposited nitrogen was sampled weekly for the three month period: rainfall volume was obtained from a rain gauge and NO_3, NH_4, and TN were measured for each weekly rainfall sample (methods described earlier).

Estimating total nitrogen deposition loads

Gaseous nitrogen dioxide and ammonia concentrations were converted to gaseous NO_2-N and NH_3-N. Total mg of gaseous N and wet TN deposition for an individual mesocosm was calculated by accounting for the mesocosm surface area exposed to atmospheric deposition and volume of rainfall for the wet deposition.

NO_2-N and NH_3-N concentrations were converted to N fluxes using a deposition velocity of 1.13 mm s^{-1} for NO_2-N (Jones et al., 2004) and 22mm s^{-1} for NH_3-N (Jones et al., 2013). Total nitrogen concentrations from weekly rainfall samples were converted to fluxes using rainfall volumes and bulked to a monthly wet deposition flux.

5.3.6 Plant responses

At the end of July species cover was recorded using visual estimates of percentage cover for each species in each mesocosm. In order to measure plant tissue chemistry four leaves from each *Carex flacca* specimen were harvested, dried for 38 hours at 30 ºC and ground using a ball mill. The samples were then analysed for Total C and total N by dry combustion using Leco Truspec CN analyser (Leco corp., St Joseph, MI, USA), with tissue nitrogen content and the carbon to nitrogen ratio calculated from this.

5.3.7 Statistical analysis

All statistical analysis was performed using Minitab v.16. Differences in nitrogen outputs (mg of N) between DIN treatments were analysed using a general linear model to test for the individual differences caused by the wet and dry treatments, nitrogen treatments and the interaction between the two.

Differences between the bi-weekly means of the total water losses in both wet and dry mesocosms from May to July were analysed by analysis of covariance. Data that proved not normally distributed (Kolmogorov-Smirnov test) were transformed using a Johnson's transformation (Johnson, 1995), which transforms the data to follow a normal distribution. Where transformation was not sufficient

to achieve assumptions of normality (24/06/2014) a non-parametric Kruskal-Wallis test was carried out.

The difference between the mean species percentage cover for each species within all treatments and *Carex flacca* tissue chemistry was analysed using a general linear model to test for the individual differences caused by the wet and dry treatments, nitrogen treatments and the interaction between the two ("water table" "nitrogen" "water table * nitrogen").

5.4 Results

A water budget calculated for mesocosms subject to wet and dry hydrological water regimes, which accounted for rainfall, groundwater inputs and overflow water outputs, showed that the dry water regime treatment was consistently maintained at 10 cm lower than the wet water regime treatment for the three month study (Fig 5.1 d).Total water losses from evapotranspiration over this period within mesocosms subject to the wet hydrological regime were 403.31 ± 6.88 mm, significantly higher than 334.04 ± 5.86 mm water losses within mesocosms subject to the dry hydrological regime. Within the first two weeks in May and last two weeks in June and July (Fig 5.1 a), water losses were significantly greater ($F= 297.85$ $df= 1$ $p= 0.000$) in mesocosms subject to a wet water regime compared to those subject to a dry water regime. Water losses were significantly greater in dry mesocosms only for the first two weeks in July. This was an artefact caused by the supplementary addition of groundwater mix (Fig 5.1 a) to all treatments following a dry spell. With no rainfall recorded from mid-June to the beginning of July (Fig 5.1 c), the water table declined for both wet and dry mesocosms (Fig 5.1 d) and as a result the water losses within the wet mesocosms were much greater than those from the other months. Following the water addition (Fig 5.1 d), water tables for both wet and dry mesocosms returned to treatment water table depths, however due to the drier sand in the dry treatments there was more uptake of water, which was required to achieve saturation to the target water level. The daily recorded temperature shows a general increase from May to July whereby the average temperature recorded (including day and night readings) was 14 °C for May, 19 °C for June and 20 °C for July.

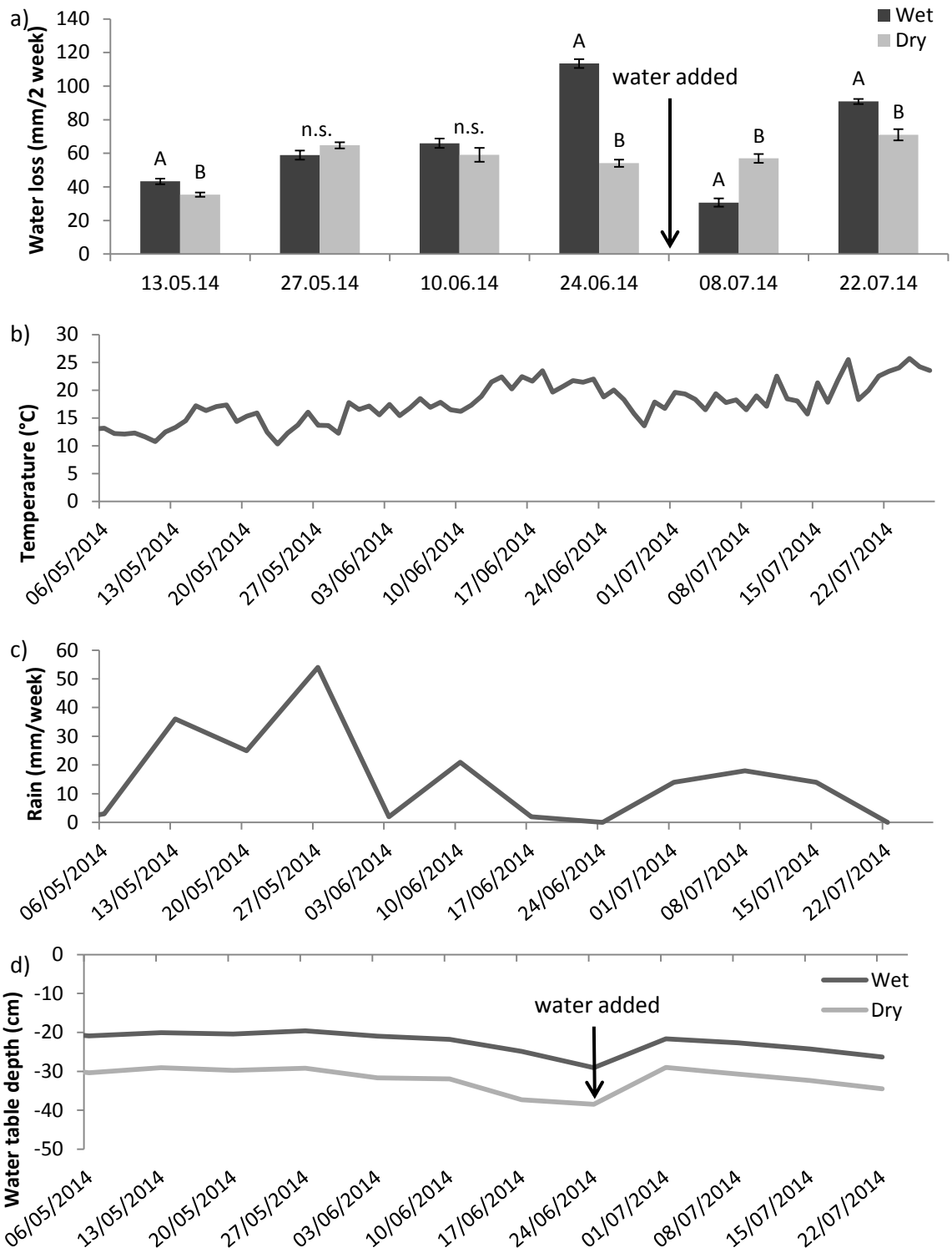


Fig 5.1 Three month time series for wet and dry treatments; a) fortnightly water loss, b) daily average temperature, c) weekly rainfall and, d) weekly water table depth. (Different letters denote significance between treatments; n.s = no significance. The black arrow indicates when 2 litres of water was added to both treatments.)

The plant responses to the wet and dry treatment, the nitrogen treatment and the interaction between the two were analysed. The percentage cover of the forb *Prunella vulgaris* (Fig 5.2 d) was significantly greater (F= 19.15 df= 1 p=0.000) within the dry treatment than the wet, whereas the *Carex flacca* sedge (Fig 5.2 b) showed a significantly greater (F= 6.81 df= 1 p=0.013) percentage cover in the wet treatment compared to the dry. There was no significant differences between the wet and dry treatments for *Leontodon autumnalis* (Fig 5.2 c) and *Carex arenaria* (Fig 5.2 a), and no influence on overall species percentage cover from the nitrogen treatment or the interaction between the wet and dry treatments and nitrogen treatments (Fig 5.2). *Carex flacca* had the greatest percentage cover within all mesoscosms compared with all other species.

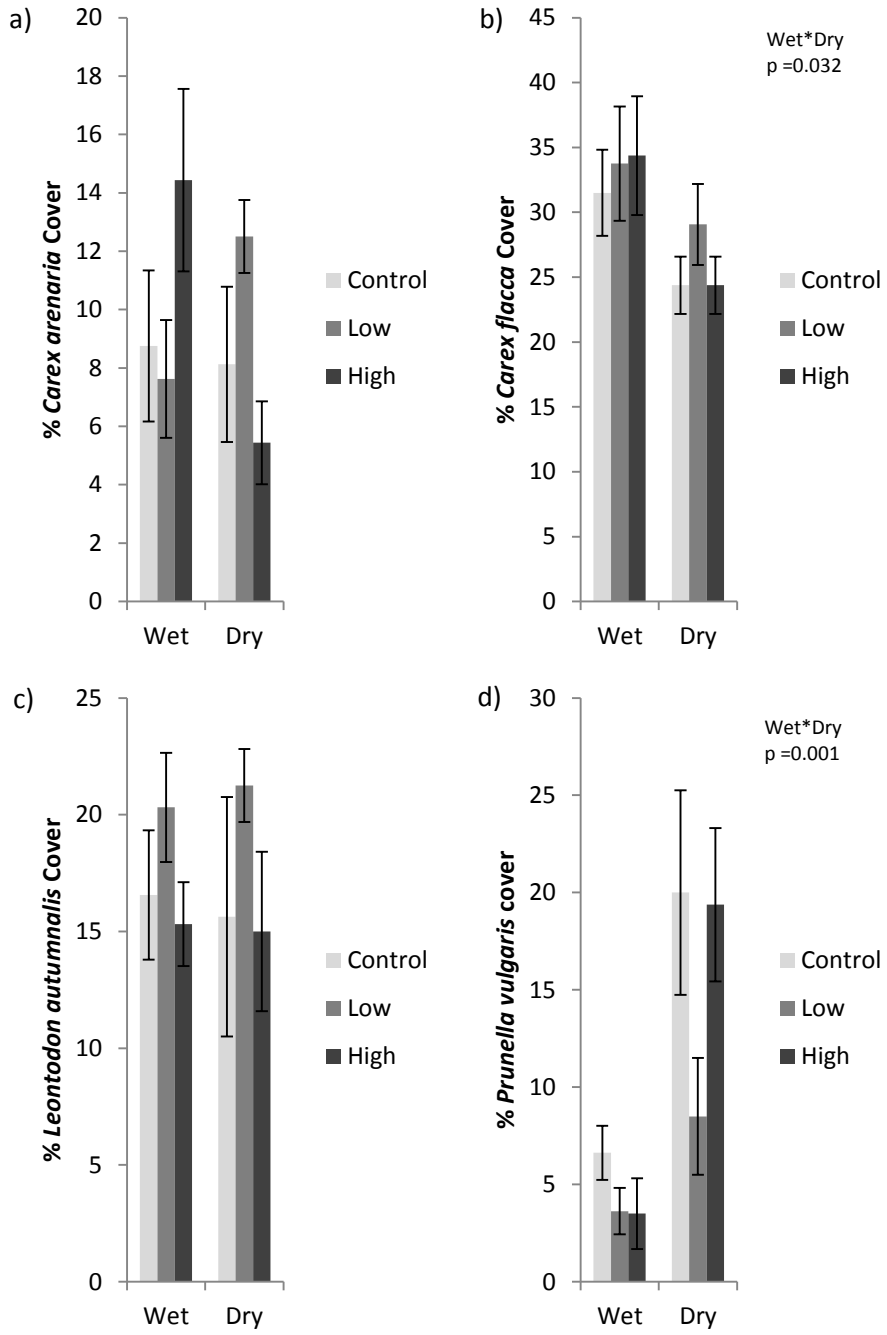


Fig 5.2 Species percentage cover in wet/dry and nitrogen treatments for a) *Carex arenaria,* b) *Carex flacca,* c) *Leontodon autumalis* and, d) *Prunella vulgaris.* No difference was found between nitrogen treatments; significant differences between wet and dry treatments are indicated.

In order to compare the effects of the experimental treatments on nutrient uptake in plants, the nitrogen and carbon content was measured within the leaves of the most successful species within the experiment, *Carex flacca*. The comparison of nitrogen treatments showed that plant tissue nitrogen of *C. flacca* was elevated in the high nitrogen treatment, with values significantly greater

(F= 3.87 df= 2 p= 0.029) than the low nitrogen treatment (Fig 5.3 a), although they were not significantly different from the control. The C:N ratio was not quite significantly different (p= 0.084) between the nitrogen treatments (Fig 5.3 b), although the C:N ratio is nonetheless noticeably lower within the high nitrogen treatment than the control and low nitrogen treatments. No difference was found when comparing the effects of the wet and dry treatment or the interaction between the wet and dry treatment with nitrogen treatment on both nitrogen content and the C:N ratio.

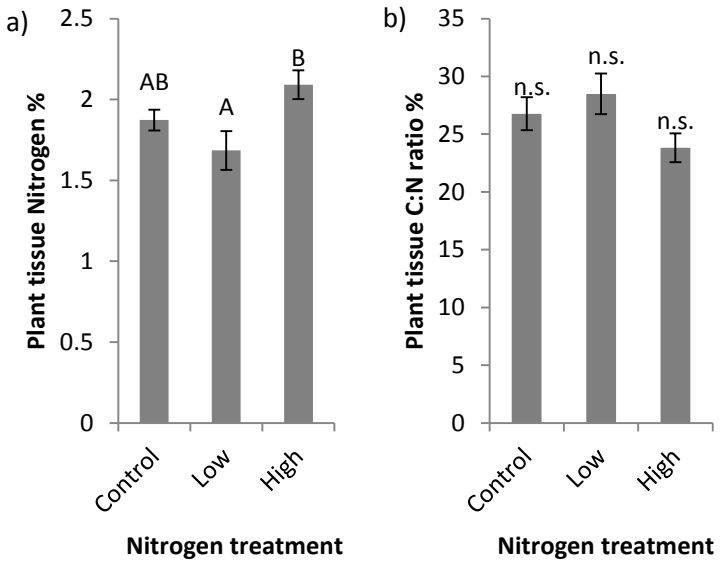


Fig 5.3 *Carex flacca* tissue composition of a) percentage plant tissue nitrogen and, b) percentage C:N ratio for all nitrogen treatments. Different letters denote significance between treatments; n.s = not significant.

The average monthly target DIN treatment concentrations for all nitrogen treatment measurements are shown within a time series (Fig 5.4). The average DIN concentrations of the treatments for the whole experimental period were maintained at (average ± standard error): Control 0.151 ± 0.170 mg l^{-1}, low 0.218 ± 0.018 mg l^{-1} and high 9.486 ± 0.370 mg l^{-1}.

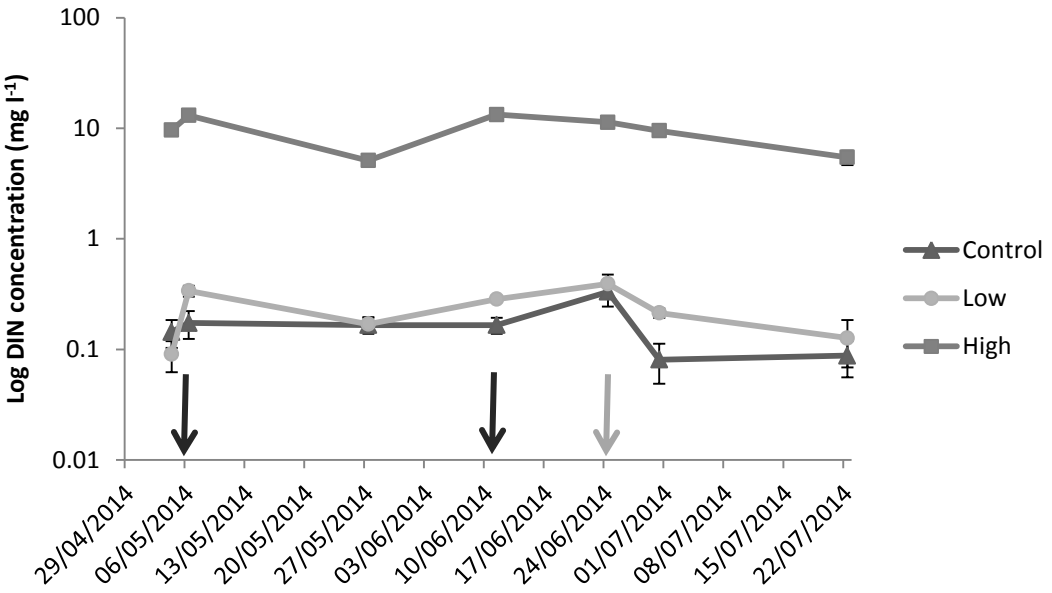


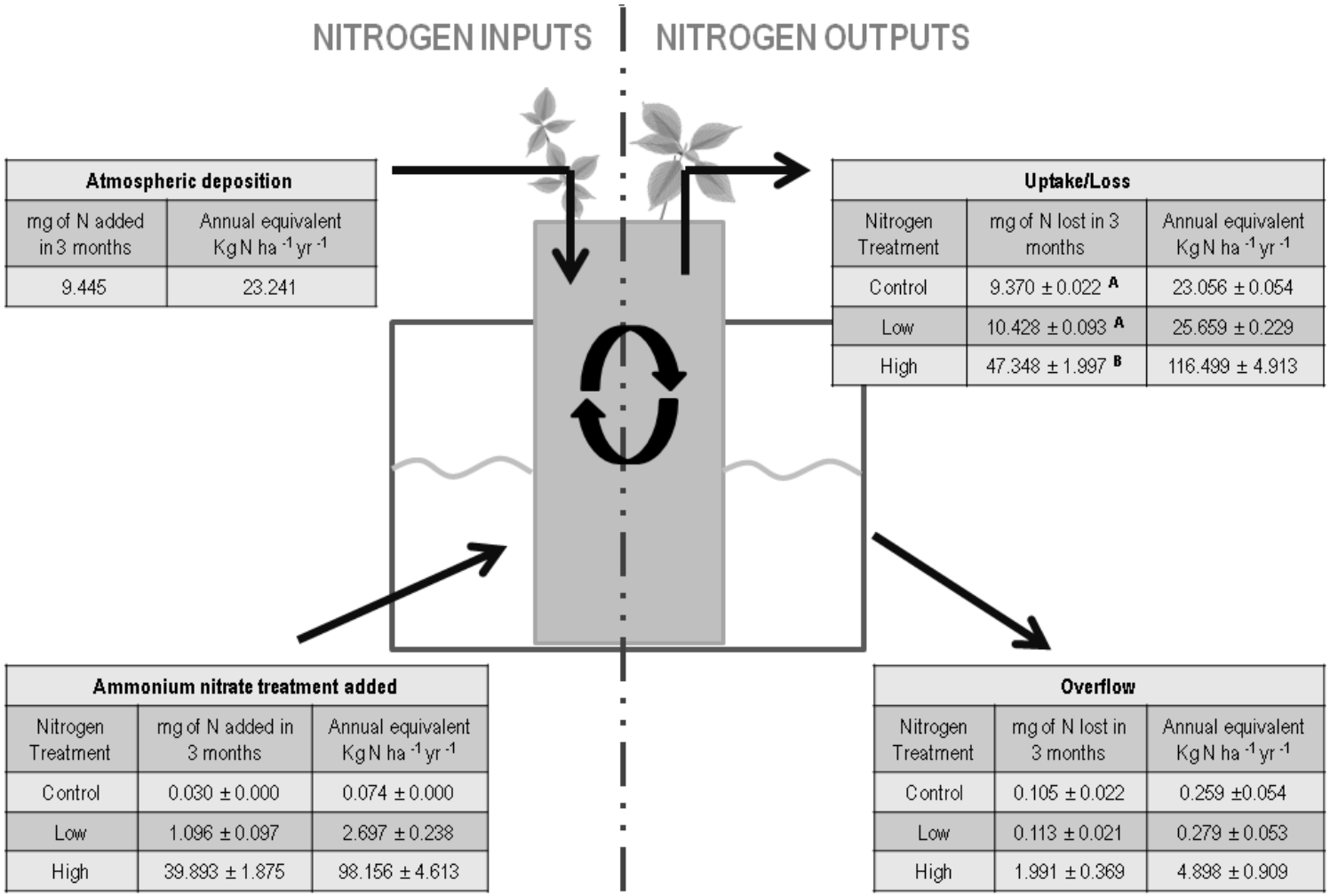
Fig 5.4 Time series of log DIN concentrations for all nitrogen treatments for the three month period. Black arrows represent when ammonium nitrate treatment was added and the grey arrow represents when both groundwater stock and ammonium nitrate treatment was added.

The separation of atmospheric deposition into wet and dry classes (Table 5.2); shows that rainfall contributes double the amount of atmospheric nitrogen inputs compared to the total dry gaseous nitrogen inputs. Very high rainfall volumes in May represented most of the wet deposition measured, so the annual equivalent is overestimated. The highest proportion of gaseous nitrogen inputs is from gaseous ammonia with a small amount contributed by nitrous oxide depositions.

Table 5.2 Total wet and dry measured atmospheric deposition inputs into individual mesocosms from May to July and calculated annual deposition from the 3 month measurements.

Atmospheric deposition		mg of N deposited in 3 months, per mesocosm	Annual equivalent (kg N ha^{-1} yr^{-1})
Wet	NO_3-N +NH_4-N	5.985	14.726
Dry	NO_2-N	0.668	1.644
	NH_3-N	2.792	6.870

The sum of nitrogen inputs and outputs for the three months is presented in Figure 5.5. This shows the quantities of N added to maintain groundwater concentrations, where quantities for the control and low DIN treatments were much lower than inputs from atmospheric deposition. The quantities in the high DIN treatment, however, were considerably higher than atmospheric deposition. As a result, the comparison of nitrogen outputs among the three DIN treatments (Fig 5.5), for the total mg of DIN uptake or losses from May to June, were significantly higher in the high nitrogen treatment than the control and low nitrogen treatments. The losses could not be separately quantified as plant, soil or microbial uptake. No significant difference was found between the control and low nitrogen treatments.



Atmospheric deposition	
mg of N added in 3 months	Annual equivalent Kg N ha $^{-1}$ yr $^{-1}$
9.445	23.241

Uptake/Loss		
Nitrogen Treatment	mg of N lost in 3 months	Annual equivalent Kg N ha $^{-1}$ yr $^{-1}$
Control	9.370 ± 0.022 ^{A}	23.056 ± 0.054
Low	10.428 ± 0.093 ^{A}	25.659 ± 0.229
High	47.348 ± 1.997 ^{B}	116.499 ± 4.913

Ammonium nitrate treatment added		
Nitrogen Treatment	mg of N added in 3 months	Annual equivalent Kg N ha $^{-1}$ yr $^{-1}$
Control	0.030 ± 0.000	0.074 ± 0.000
Low	1.096 ± 0.097	2.697 ± 0.238
High	39.893 ± 1.875	98.156 ± 4.613

Overflow		
Nitrogen Treatment	mg of N lost in 3 months	Annual equivalent Kg N ha $^{-1}$ yr $^{-1}$
Control	0.105 ± 0.022	0.259 ±0.054
Low	0.113 ± 0.021	0.279 ± 0.053
High	1.991 ± 0.369	4.898 ± 0.909

Fig 5.5 Conceptual diagram indicating total nitrogen inputs and outputs from May to July and calculated annual equivalent kg N ha^{-1} yr^{-} from the 3 month measurements. Values are expressed as mean ± standard error and values denoted with the same letter are not significantly different.

5.5 Discussion

Results of this study show that high DIN groundwater concentrations increase nitrogen uptake by vegetation and losses (through plant, soil and microbial uptake) within dune slack habitats, however groundwater DIN concentrations ≤ 0.2 mg l^{-1} had no effect on nitrogen uptake and losses. A water table lowered by 10 cm showed higher water losses in the wetter dune slack mesocosms and were also seen to affect percentage plant cover in a forb and sedge species.

5.5.1 Water budget

Previous studies have shown that annual water losses through evapotranspiration are greater in wet dune slacks than dry slacks. Within wet slacks where the water table fluctuates from +20 to -90 cm, evapotranspiration losses are estimated at 550 mm a^{-1}, compared to 330 mm a^{-1} in drier dune slacks where the water table fluctuates from -35 to -170 cm (Stratford et al., 2007). In this study, the hydrological regime of both the wet and dry treatments more closely mimic that of the wet dune slack described by Stratford et al. (2007), although water tables lowered by 10 cm still caused water losses to be significantly greater within the wet treatment than the dry treatment over the three month period. These findings draw attention to site specific water-balanced models created for dune slack habitats (e.g. Clarke and Ayutthaya, 2010), and suggest that improvements that consider such losses from small fluctuations could be implemented. Overall, these findings are comparable to those of Stratford et al. (2007), which suggest that water losses within natural dune slack systems are likely to decrease with lowered water tables from climate change (Clarke and Ayutthaya, 2010).

5.5.2 Botanical responses

Previous work has shown that dune slacks subject to long-term nutrient contamination concentrations, at concentrations as low as 0.2 mg l^{-1} of DIN within the groundwater, had an ecological impact on dune slack species composition (Rhymes et al., 2014). However, while the current study used similar low concentrations to that of Rhymes et al. (2014), no changes in species percentage cover and tissue chemistry were found. This is likely to be due to the short duration of the experiment, as such small concentrations are only likely to have an ecological impact following long-term exposure. This suggests that dune systems may recover with limited or no ecological impacts from a single, short-term and low concentration DIN groundwater contamination event. Long-term exposure however, is likely to lead to biological impacts. Species percentage cover was also unaffected by the high nitrogen treatment, as it is likely that soils were limited by phosphorus, since phosphorus and nitrogen can be found to be co-limiting (Lammerts et al., 1999).

It is well documented that species composition and distribution within dune slack habitats are primarily influenced by water table depth (Curreli et al., 2013, Willis et al., 1959). We found that the growth of *Prunella vulgaris* was positively influenced by the drier soils (Fig 5.2 a). The opposite response was recorded for *Carex flacca* (Fig 5.2 c), which is largely consistent with the UK National vegetation classification (Rodwell et al., 2000), whereby the SD16 drier slack community comprises of relatively lower *Carex flacca* and higher *Prunella vulgaris* and the wettest subtype slack community of SD14, SD14b, is characterised by higher *Carex flacca* and lower *Prunella vulgaris* (Rodwell et al., 2000). This study indicates the vulnerability of individual dune slack species to changes in water tables as small as 10 cm. With only 40 cm differences in water table depth separating the drier from the wetter dune slack communities, and the increasing threat of dropping water table depths due to climate change (Curreli et al., 2013), dune slack community structures are likely to change from wetter SD15/14 to drier SD16 communities (Rodwell et al., 2000, Curreli et al., 2013). These changes will be intensified (Bakker et al., 2006, Grootjans et al., 1996) and could result in extreme shifts to grassland SD8 communities, when sand dune sites are subject to drainage or groundwater abstraction.

With regard to the effects of increased nitrogen loads on plant tissue nitrogen content, increases in atmospheric ammonium deposition have been seen to increase tissue nitrogen content within several dry dune species (Jones et al., 2013). The average plant tissue nitrogen content for *Carex flacca* in this study was above 1.5 % across all three nitrogen treatments; in comparison with results from Jones et al. (2013), our results are representative of species subject to 22 kg ha $^{-1}$ yr $^{-1}$ of ammonia fumigation. This suggests that nitrogen sourced from both atmospheric and groundwater inputs are being stored and utilised by the vegetation as a nitrogen source within all nitrogen treatments, with tissue nitrogen content being greater where nitrogen availability in groundwater is greatest.

5.5.3 Nitrogen budget

Our annual calculated atmospheric deposition load of 23.24 kg N ha^{-1} yr^{-}, which accounts for summer months only, is slightly higher than the modelled critical load ranges within the same location and for wet dune slack habitats of 10-20 kg N ha^{-1} yr^{-} (Air Pollution Information System, Accessed on 22/01/15).The experiment was conducted within an urban area where dry atmospheric loads are 47% higher than non-urban areas (Bettez and Groffman, 2013), nevertheless, the atmospheric deposition loads within this study and across much of Europe still exceed the annual critical load defined for dune slacks 10-15 kg N ha^{-1} yr^{-1} (Bobbink and Hettelingh, 2010).

The nitrogen uptake/loss term calculated is probably a combination of nitrogen uptake by the plants, soil and microbes, and losses through denitrification, however, the losses could not be separately quantified in this experiment. The use of 15N labelling would allow for this and is recommended for future studies. Nitrogen losses were not affected by the differing water table regimes, which is likely due to the soils and plants within both treatments having equal accessibility to groundwater nitrogen due to capillary processes, which can carry water 45cm above the water table (Ranwell, 1959), and due to deeper rooting depths observed within drier slack communities (Rhymes et al., 2014). With respect to the nitrogen treatment losses, the increased availability of groundwater DIN (in this case ammonium nitrate) can: 1) cause nitrogen uptake within plants, with nitrogen subsequently stored in plant tissue and processed (Plassmann et al., 2009); 2) cause direct binding of ammonium by the soil; and 3) increase denitrification rates whereby nitrogen is reduced and lost as nitrogen gases (Merrill and Zak, 1992). Indeed, high denitrification rates have been found to persist for longer durations with increased groundwater nitrogen availability, increasing the nitrogen losses through greenhouse gas emissions (Rhymes et al., 2015, in prep.). As a result of the high nitrogen treatment increasing the availability of DIN to the plants, soil and microbes, these processes are likely to have caused nitrogen losses to be significantly greater within the high nitrogen treatment than both the control and low nitrogen treatments.

The former study carried out within a natural dune system (Rhymes et al., 2014) found increased soil nitrogen concentrations, an indication of nitrogen uptake, within dune slacks subject to DIN groundwater concentrations as low as 0.2mg l^{-1}. It should be noted, however, that this study only allowed for the quantification of N losses, representative of N uptake by the plant and soil system or through denitrification. We found no differences in potential nitrogen losses and uptake in the low nitrogen treatment compared with the control (i.e. no ammonium nitrate addition). The mesocosms were however static with no groundwater flow and the equivalent of nearly 3 kg N ha^{-1} yr^{-} as well as the comparatively high background atmospheric deposition over a 3 month period was required to maintain groundwater DIN concentrations of 0.2 mg l^{-1}. We conclusively demonstrate that within 3 months, dune species uptake nitrogen although this did not alter plant growth. Longer term effects, however, could be mediated by other processes, such as nitrogen turnover rates or the accumulation of nitrogen within the soils, before ecological impacts are apparent. This implies that fluxes of uptake and losses within a natural sand dune system subject to low concentrations of contamination may be quite high, with our recommendation being to calculate these in the field at sites with known impacts.

5.6 Conclusions

This study has established that nitrogen storage uptake/loss within dune slack habitats is increased with increased groundwater DIN concentrations, with evidence of nutrient uptake by vegetation. This highlights the necessity to include the contribution of groundwater nitrogen loads to the overall critical nitrogen loads for dune slack habitats (Bobbink and Hettelingh, 2010). We suggest that additional work is required to investigate the storage, microbial processing and greenhouse gas contribution of nitrogen within the soil. Moreover, other technical experimental approaches are also needed on all ecosystem processes to separate the contribution of nitrogen from groundwater nitrogen inputs and atmospheric inputs. This study also highlights the vulnerability of dune slack communities to climate change. Changes in plant species cover due to a 10 cm change in water table depth emphasises the necessity to consider the potential impacts of groundwater abstraction on water tables and therefore on the botanical composition of dune slacks, and to implement conservational management plans.

5.7 References

BAKKER, C., VAN BODEGOM, P. M., NELISSEN, H. J. M., ERNST, W. H. O. & AERTS, R. 2006. Plant responses to rising water tables and nutrient management in calcareous dune slacks. *Plant Ecology,* 185**,** 19-28.

BERENDSE, F., LAMMERTS, E. & OLFF, H. 1998. Soil organic matter accumulation and its implications for nitrogen mineralization and plant species composition during succession in coastal dune slacks. *Plant Ecology,* 137**,** 71-78.

BETTEZ, N. D. & GROFFMAN, P. M. 2013. Nitrogen deposition in and near an urban ecosystem. *Environmental Science & Technology,* 47**,** 6047-6051.

BOBBINK, R. & HETTELINGH, J.-P. Review and revision of empirical critical loads and dose-response relationships. Proceedings of an expert workshop, Noordwijkerhout, 2010. 23-25.

CAMARGO, J. A. & ALONSO, A. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International,* 32**,** 831-849.

CLARKE, D. & AYUTTHAYA, S. S. N. 2010. Predicted effects of climate change, vegetation and tree cover on dune slack habitats at Ainsdale on the Sefton Coast, UK. *Journal of Coastal Conservation,* 14**,** 115-125.

CURRELI, A., WALLACE, H., FREEMAN, C., HOLLINGHAM, M., STRATFORD, C., JOHNSON, H. & JONES, L. 2013. Eco-hydrological requirements of dune slack vegetation and the implications of climate change. *Science of the Total Environment,* 443**,** 910-919.

DAVY, A., HISCOCK, K., JONES, M., LOW, R., ROBINS, N. & STRATFORD, C. 2010. Protecting the plant communities and rare species of dune wetland systems: ecohydrological guidelines for wet dune habitats. Phase 2. Protecting the plant communities and rare species of dune wetland systems. Bristol, UK: Environment Agency.

FOWLER, D., O'DONOGHUE, M., MULLER, J., SMITH, R., DRAGOSITS, U., SKIBA, U., SUTTON, M. & BRIMBLECOMBE, P. 2005. A chronology of nitrogen deposition in the UK between 1900 and 2000. *Water, Air, & Soil Pollution: Focus,* 4**,** 9-23.

GALLOWAY, J. N. & COWLING, E. B. 2002. Reactive nitrogen and the world: 200 years of change. *Ambio,* 31**,** 64-71.

GROOTJANS, A., ADEMA, E., BEKKER, R. & LAMMERTS, E. 2004. Why coastal dune slacks sustain a high biodiversity. *Coastal Dunes.* Springer.

GROOTJANS, A. P., SIVAL, F. P. & STUYFZAND, P. J. 1996. Hydro-geochemical analysis of a degraded dune slack. *Vegetatio,* 126**,** 27-38.

JOHNSON, D. H. 1995. Statistical sirens: the allure of nonparametrics. *Ecology*, 1998-2000.

JONES, L., NIZAM, M., REYNOLDS, B., BAREHAM, S. & OXLEY, E. 2013. Upwind impacts of ammonia from an intensive poultry unit. *Environmental Pollution,* 180**,** 221-228.

JONES, M. L. M., WALLACE, H. L., NORRIS, D., BRITTAIN, S. A., HARIA, S., JONES, R. E., RHIND, P. M., REYNOLDS, B. R. & EMMETT, B. A. 2004. Changes in vegetation and soil characteristics in coastal sand dunes along a gradient of atmospheric nitrogen deposition. *Plant Biology,* 6**,** 598-605.

LAMMERTS, E., PEGTEL, D., GROOTJANS, A. & VEEN, A. V. D. 1999. Nutrient limitation and vegetation changes in a coastal dune slack. *Journal of Vegetation Science,* 10**,** 111-122.

MERRILL, A. G. & ZAK, D. R. 1992. Factors controlling denitrification rates in upland and swamp forests. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere,* 22**,** 1597-1604.

PLASSMANN, K., EDWARDS-JONES, G. & JONES, M. L. M. 2009. The effects of low levels of nitrogen deposition and grazing on dune grassland. *Science of the Total Environment,* 407**,** 1391-1404.

PROVOOST, S., JONES, M. L. M. & EDMONDSON, S. E. 2011. Changes in landscape and vegetation of coastal dunes in northwest Europe: a review. *Journal of Coastal Conservation,* 15**,** 207-226.

RANWELL, D. 1959. Newborough Warren, Anglesey: I. The dune system and dune slack habitat. *The Journal of Ecology***,** 571-601.

REMKE, E., BROUWER, E., KOOIJMAN, A., BLINDOW, I., ESSELINK, H. & ROELOFS, J. G. 2009. Even low to medium nitrogen deposition impacts vegetation of dry, coastal dunes around the Baltic Sea. *Environmental Pollution,* 157**,** 792-800.

RHYMES, J., JONES, L., WALLACE, H., JONES, T., DUNN, C. & FENNER, N. 2015. Groundwater nutrients and water levels alter nitrogen and carbon processing in dune slack soils. *In preparation*.

RHYMES, J., WALLACE, H., FENNER, N. & JONES, L. 2014. Evidence for sensitivity of dune wetlands to groundwater nutrients. *Science of the Total Environment,* 490C**,** 106-113.

RODWELL, J., DRING, J., AVERIS, A., PROCTOR, M., MALLOCH, A., SCHAMINÉE, J. & DARGIE, T. 2000. Review of coverage of the National Vegetation Classification. *Report-Joint Nature Conservation Committee*.

STRATFORD, C., RATCLIFFE, J., HUGHES, A. G., ROBERTS, J. & ROBINS, N. S. Complex interaction between shallow groundwater and changing woodland, surface water, grazing and other influences in partly wooded duneland in Anglesey, Wales. Proceedings of the CDXXXV congress international association of hydrogeologists: groundwater and ecosystems. International Association of Hydrogeologists, 2007. 1-10.

STRATFORD, C. J., ROBINS, N. S., CLARKE, D., JONES, L. & WEAVER, G. 2013. An ecohydrological review of dune slacks on the west coast of England and Wales. *Ecohydrology,* 6**,** 162-171.

SYSTEM, A. P. I. Accessed on 22/01/15. http://www.apis.ac.uk/.

VAN DER LAAN, D. 1979. Spatial and temporal variation in the vegetation of dune slacks in relation to the ground water regime. *Vegetation,* 39**,** 43-51.

WILLIS, A., FOLKES, B., HOPE-SIMPSON, J. & YEMM, E. 1959. Braunton Burrows: the dune system and its vegetation. *The Journal of Ecology***,** 249-288.

CHAPTER 6: **Synthesis and Conclusions**

6.1 Introduction

The increased availability of nitrogen and emerging threats from climate change are a major concern for dune slack habitats. The impacts of these pressures on these low nutrient habitats and specific hydrological requirements are poorly understood. Research efforts have predominantly focused either on the impacts of atmospheric nitrogen deposition or on water table depths, with no studies to date investigating the effects of groundwater nitrogen contamination, at what concentrations impacts occur along with the synergistic effects from nitrogen pollution and water table depth.

As outlined in chapter 1, the over-arching research aims for this thesis were;

- To examine the impacts of nitrogen groundwater contamination on dune slack soil chemistry and vegetation and at what concentrations such impacts occur.
- To investigate the impacts from the interaction of both climate change and groundwater nitrogen contamination on soil processes and the fate of that nitrogen.
- To utilise a combination of inorganic analytical techniques to evaluate the potential sources and pathways of groundwater nutrients and contamination to sand dune sites.

With these key research focuses this chapter summarises the existing knowledge and discusses the key findings from this study and their relevance to the wider dune slack science knowledge, management and conservation. The limitations of the study are also considered with suggestions for future research.

6.2 Impacts from groundwater nitrogen contamination and climate change alone and in synergy.

Many aquatic ecosystems are under threat from groundwater contamination and a global assessment concluded that concentrations above 0.5-1.0 mg l^{-1} of TN could lead to eutrophication (Camargo and Alonso, 2006). Broadly consistent with these concentrations, a collation of groundwater chemistry from dune slacks states that values >1 mg l^{-1} of DIN indicates probable nutrient contamination and merit concern, whilst concentrations of 0.2 mg l^{-1} or below are not a cause for concern (Davy et al., 2010). The only previous evidence of impacts from highly eutrophic river water around dune drinking water infliltration ponds was carried out in the Netherlands (Meltzer and Van Dijk, 1986), therefore, this is the first study to identify *in situ* ecological impacts on

dune slack ecology from groundwater contamination concentrations as low as 0.2 mg l^{-1} of DIN, within chapter 2. While a mesocom investigation, chapter 4 and 5, did not show ecological impacts at these concentrations, it did show uptake and processing of extra nitrogen within sand dune systems. We investigated the *in situ* responses of vegetation assemblages and soil chemistry at these low concentrations, whilst *ex situ* experimental manipulations investigated species success and soil processes at various groundwater DIN concentrations including the 0.2 mg l^{-1} along with synergistic impacts from groundwater nitrogen contamination and climate change. These are discussed in more detail below.

6.2.1 Vegetation assemblages

Sand dune vegetation responses to nitrogen contamination have predominantly focused on impacts from increased atmospheric nitrogen deposition. Previous studies have shown increases in biomass (Van den Berg et al., 2005, Plassmann et al., 2009) when the 10 -15 kg N ha^{-1} yr^{-1} critical load of inputs from atmospheric deposition (Bobbink and Hettelingh, 2010) are exceeded, in some cases such impacts are also observed at lower deposition loads (Jones et al., 2013). A review of wet dune slack fertilisation experiments (Lammerts and Grootjans, 1997), showed a common decrease in characteristic basiphilous pioneer species, such as *Anagallis tenella*. Similar results were also found in this study, chapter 2, where the impacts of groundwater nitrogen contamination were explored within a sand dune site exposed to a nutrient gradient. We found that within dune slacks subject to higher groundwater nitrogen concentrations nitrogen influenced species composition independently of water table depth and soil development, causing an increase in nitrophilous species, such as *Rubus caesius* and *Potentilla reptans* and a decrease in basipholous species (Fig 2.4 and Table 2.3). These responses were recorded at concentrations as low as 0.2 mg l^{-1} of DIN, which is currently a concentration described as 'no cause for concern' on dune slack habitats (Davy et al., 2010) and much lower than the proposed wet dune groundwater thresholds of 3 mg l^{-1} of N by UKTAG (2014). In view of these results, where currently the consensus is, we strongly recommend that the dune slack nitrogen critical load described by (Bobbink and Hettelingh, 2010), which currently only accounts for atmospheric inputs, is reconstructed to consider inputs of N from groundwater as part of the overall nutrient critical load.

6.2.2 Response of individual species

Within chapter 2, in situ plant species composition was primarily controlled by water table depth and water table fluctuations (Fig 2.4 a & b) which is in broad agreement with the literature (e.g. Lammerts et al., 2001). Groundwater nitrogen contamination, however, independently influenced

the vegetation composition too. A mesocosm investigation, chapter 5, demonstrated that the success of two dune slack species was similarly affected by water table depth, however, the added influence of groundwater nitrogen contamination was not observed (Fig 5.2). As the species within the mesocosm investigation were only subject to their treatments for a short period of time, unlike the vegetation assemblages observed in situ, in chapter 2; it is possible that longer term groundwater nitrogen contamination will eventually alter the percentage cover by both water table depth and groundwater nitrogen contamination; results from chapter 4 and 5 show both direct uptake of nitrogen into dune systems. This uptake of nitrogen into these systems is subsequently mediated by soil processes and uptake by the vegetation. The lack of vegetation responses to groundwater nitrogen contamination in chapter 5, could have also been due to the limitation of other nutrients, as many dune slack species have been seen to be primarily-limited by nitrogen, however, are secondarily-limited by phosphorus (Lammerts and Grootjans, 1997).

6.2.3 Vegetation nitrogen uptake

Groundwater nutrient uptake by vegetation and storage within the plant tissue can occur when the roots are in contact with groundwater. Within sand dune systems, even when the water table is 45 cm below the rooting zone, roots still have access to the groundwater as water is carried up by a capillary process (Ranwell, 1959). The impacts of lowered water tables, by climate change or groundwater abstraction, on nutrient uptake by dune slack vegetation, have not previously been examined. Within chapter 2 the assessment of the rooting zone between wet and dry slacks, with around 25 cm difference in water table depth (Fig 2.3), suggested that the water table was likely to be a major factor controlling rooting depth. The main rooting zone within the wetter dune slack community was in the shallower sections of the soil profile, 0 to -40 cm, compared with the deeper 0 to -60 cm zone within the drier slacks (Fig 2.3). The differing water table regimes, however, within the dry and wet slacks, along with soil capillary processes and differences in rooting zones, suggests that both wet and dry slacks are equally vulnerable from groundwater nitrogen contamination because the roots extend deeper in the dry slacks and therefore still have access to the lower water tables. The findings within chapter 5 agree with this hypothesis, as we found that a 10 cm difference in water table depth had no effect on plant nitrogen uptake, measured as plant tissue nitrogen content within *Carex flacca*. This could, however, be as a result of the minimal 10 cm difference in water table depth and further investigations would be required, with larger differences in water table depths to explore such hypothesis.

In previous studies, an increase in atmospheric ammonia deposition has been seen to increase tissue nitrogen content within several dry dune species (Jones et al., 2013). In chapter 5 where various different groundwater nitrogen treatments (0, 0.2 and 10 mg l^{-1}) along with a high atmospheric nitrogen deposition concentration (Fig 5.2) was employed, we found that plant tissue nitrogen content increased within the high 10 mg l^{-1} compared to the low 0.2 mg l^{-1} DIN groundwater treatment (Fig 5.5 a). These findings, from both chapter 2 and 5, therefore suggest that nutrient uptake is likely to only be affected by increased nitrogen availability, rather than a combination of availability and water table depth within wet and dry dune slack habitats. The impacts on nutrient uptake from predicted lowering of water table depths by up to 100 cm by 2080 (Clarke and Ayutthaya, 2010) were not explored within this study. It is likely, however, that the lowering of water tables to such extreme depths within dune slack habitats, will alter vegetation assemblages into dune grasslands habitats (Curreli et al., 2013). In turn, these areas will no longer be inhabited by pioneer dune slack species, which will alter the rooting depths and subsequently alter nutrient uptake by the vegetation.

6.2.4 Soil nitrogen content and denitrification

Within the *in situ* study, chapter 2, soil nitrate and nitrite concentrations were significantly higher within dune slacks subject to higher concentrations of groundwater nitrogen contamination (Table 2.2). To the contrast Jones et al. (2004) found no effect on dune slack soil nitrogen content with increased atmospheric deposition loads. Similarly, in chapter 4 where mesocosms were subject to short term groundwater nitrogen contamination, soil chemistry was unaffected (Fig 4.3 a,b & c). This suggests that the impact from long term groundwater nitrogen contamination, as seen within our *in situ* study (chapter 2) is potentially as a result of the increase in plant tissue nitrogen content caused by increased groundwater nitrogen, as seen in chapter 4, which subsequently returns to the soil as litter fall (Berendse et al., 1998) and is directly bound as ammonium. This implies that these responses are likely to intensify year after year if biomass increases, which has been observed within dune vegetation subject to increased atmospheric nitrogen deposition (Van den Berg et al., 2005, Plassmann et al., 2009).

The chapter 4 investigation in which soil biogeochemistry was unaffected is likely to be as a result of the short duration in which mesocosms were subject to groundwater nitrogen contamination. It is likely that the nitrogen contamination is mediated by the immediate uptake of nitrogen by the vegetation and microbial community. This is supported within our study, where increases in groundwater nitrogen concentrations caused plant tissue nitrogen content to increase (chapter 5,

Fig 5.3 a) and caused a clear increasing trend of N_2O production (chapter 4, Fig 4.6) probably as a result of denitrification activity (chapter 4). Since soil nitrogen accumulation, from long term groundwater nitrogen contamination, was observed in chapter 2, yet wasn't from short term contamination in chapter 4; there is suggestion that the uptake of nitrogen by denitrification and vegetation (identified in chapter 4 and 5) may reach a threshold, following a continuation of nitrogen contamination, which could eventually cause the accumulation of nitrogen within soils. This hypothesis could be explored further within a similar experimental design to chapter 4 and 5, however exposing microcosms and mesocosms to groundwater nitrogen contamination for multiple growing seasons.

Even though the mesocosm soil biogeochemistry was unaffected by short term groundwater nitrogen inputs in chapter 4, the manipulation of water table depths caused wetter dune slack soils to have significantly lower nitrate, nitrite and DIN concentrations (Fig 4.3 a, b, & c) than within the drier soils, where the drier soils were only subject to a 10 cm lower water table depth. This may have been as a result of increased denitrification rates, where NAG enzyme activity rates were greater within the wetter soils (Fig 4.4 a), consistent with the favourable anaerobic conditions for denitrification to occur (Berendse et al., 1998). Together, findings from chapter 2, 4 and 5 suggest that long term groundwater nitrogen contamination within sand dune systems will increase nitrogen content within soils, whilst the lowering of water tables from climate change or other human activities will intensify these impacts, by reducing denitrification rates, and subsequently will cause further accumulation of nitrogen within dune slack soils.

6.2.5 Soil carbon content and carbon processing

The impacts from groundwater nitrogen contamination alone on dune slack soil carbon processing were investigated within a microcosm experiment in chapter 4. The effect of nitrogen on POX activity (an enzyme involved in the degredation of phenolic material) was found to increase with increasing contamination (Fig 4.1 c). In support similar responses were observed within peatland soils subject to atmospheric nitrogen (Bragazza et al., 2006). This suggests that dune slack soil decomposition rates which are regulated by POX activity are sensitive to groundwater nitrogen contamination, where increased nitrogen contamination increases decomposition rates, causing losses of carbon.

In chapter 4, we also investigated the effects of groundwater nitrogen contamination along with lowered water tables on dune slack mesocosms, we found that groundwater nitrogen contamination had no effect on all carbon related soil processes. The manipulation of water tables, however,

caused higher concentrations of total carbon within soils subject to a 10 cm higher water table depth. This is likely to be due to decreased decomposition rates within wetter soils, which is consistent with decreased BG activity within the wet treatment (Fig 4.4 b). In broad agreement with the literature (e.g. Whalen, 2005), methane production was greater within wetter dune slack soils in all seasons measured (Fig 4.5 b), which was expected, due to methane production primarily being an anaerobic process (Segers, 1998, Whalen, 2005). Additionally, the lower water table depth within the dry treatment increases the oxic zone within the soil profile, this encourages methanotrophic bacterial processes, which essentially limits the amount of methane released to the atmosphere (Pearce and Clymo, 2001).

Overall our findings within chapter 4 suggest that dune slack carbon processes are predominantly sensitive to water table depth, with significant results from just a 10 cm drop in water table. This study suggests that decomposition rates will increase within dune slacks with lowered water tables, and potentially cause soil decomposition to accelerate from groundwater nitrogen contamination.

6.2.6 Dune slack nitrogen budget

In chapter 5 we established that nitrogen storage uptake/loss within dune slack habitats is increased with increased groundwater DIN concentrations (Fig 5.5). When referring to nitrogen uptake/loss within this study, we make the assumption that this includes the uptake by plants, soil and microbes and losses through denitrification. These processes could not be separately identified within this study and this would require the use of labelled ^{15}N techniques within a similar experimental design. The fate of nitrogen within dune slacks is poorly understood, yet our findings in both chapter 4 and 5 show evidence of increased nutrient uptake by vegetation and increased losses through denitrification within dune slack soils subject to nitrogen groundwater contamination. Additional losses of nitrogen within dune slacks could include coupled nitrification-denitrification where slacks are inhabited by plant species capable of radial oxygen loss (ROL) (Fig 6.1 a). The presence of such species, such as *Littorella uniflora* and *Schoenus nigricans,* increases denitrification rates and subsequently lowers soil nitrogen availability (Adema et al., 2002, Adema et al., 2005). Furthermore, Adema et al. (2002) suggests that litter accumulation increases soil organic matter and therefore increases the internal dune slack nutrient cycle (Fig 6.1 b). The understanding of these ecosystem processes is crucial in the conservation of dune slack habitats.

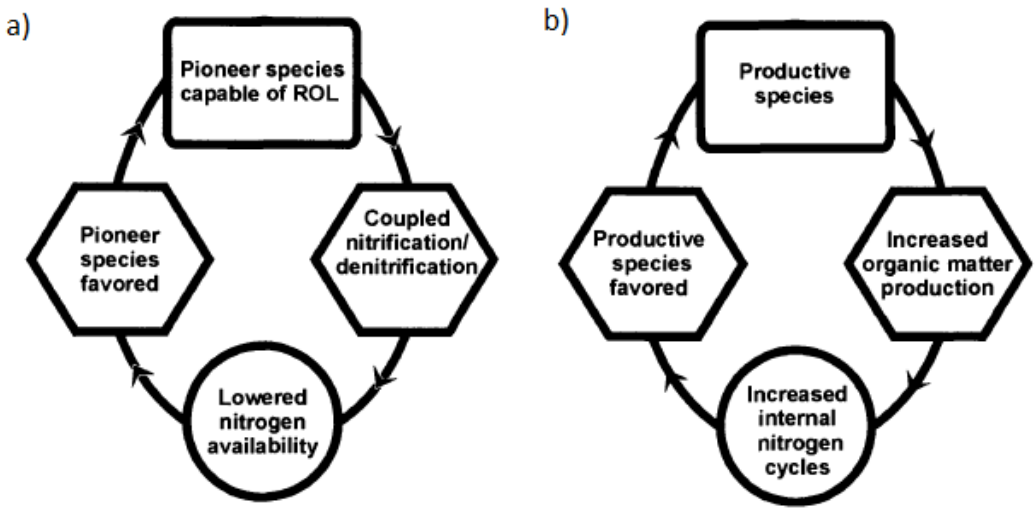


Fig 6.1 Two possible feedback mechanisms that can occur in wet dune slack a) Enhanced nitrogen loss b) Nutrient accumulation in internal cycle. Extracted from Adema et al. (2002)

6.3 Sourcing and identifying pathways of nitrogen contamination

The suggested ecological impacts found within this study suggest that groundwater nitrogen contamination alone, and in synergy with climate change, is likely to have a detrimental impact on dune slack ecology. This highlights the importance of identifying site specific nitrogen contamination inputs and their pathways, to reduce and prevent future inputs and to inform appropriate conservation management plans. The source type or types can significantly vary between sites (See Fig 1.2 for various nitrogen diffuse and point sources) and spatially vary within individual sites. Within this study (chapter 3), we used a combination of chemical, fluorescent and microbial techniques to identify nutrient sources and pathways from fertilisers and grazers cheaply. The investigation was carried out at the Aberffraw sand dune site, in which we had already identified ecological impacts from groundwater contamination in chapter 2. The combination of techniques we used allowed for the differentiation between input pathways of streams, run off events, underlying groundwater nutrient gradients and on-site grazing inputs (Fig S1), whilst also confirming the nature of sources within each pathway, as either fertilisers or faecal matter from grazers. The full potential of the fluorescence technique, however, to differentiate between livestock sources (e.g. sheep, cattle, pigs) was not explored (Baker, 2002).That said these techniques still proved extremely useful in identifying nutrient contamination sources, pathways and pressures and outlined areas on site

most at risk; in turn this information could inform appropriate site specific management plans. Such techniques could be implemented within other wetland habitats and for other applications; such as tracking faecal sources within bathing waters and fisheries zones, as currently the standard enumeration of FIB does not distinguish between human or other animal sources of contamination, and methods that do so are expensive (Field and Samadpour, 2007).

6.4 Sand dune systems provide an ecosystem service

The findings from chapter 4 and 5 show strong evidence of sand dune systems attenuating groundwater nitrogen contamination through denitrification processes, vegetation uptake and soil storage. This implies that the contamination gradient found in both chapter 2 and 3, is likely to be as a result of sand dune systems processing and storing nitrogen contamination travelling through the Aberffraw sand dune site via multiple pathways (See Chapter 3). These processes could be described as a valuable ecosystem service, as an ecosystem function become an ecosystem service when humans decipher them into valuable processes, materials and products (Nelson et al., 2013). In this case sand dune systems attenuate groundwater nutrient contamination prior to leaching into other habitats and waters. This may include the prevention of coastal water eutrophication, where eutrophication can lead to increased frequencies and magnitudes of phytoplankton blooms, which in some cases are toxic and harmful to the human health (Paerl, 1997).

Not all UK bathing sites produce full compliance with microbial standards (Crowther et al., 2002) because of the seepage of untreated sewage and increased run off and infiltration from agricultural land. However, the infiltration of storm-water through sand before reaching bathing waters provides an ecosystem service, and has seen to have a 97% bacteria catch rating (Price et al., 2013). Following detecting of faecal contamination in chapter 3, we attempted to detect the presence of a pathogenic gene gradient from faecal origin at Aberffraw sand dune site (Appendix VI), however we were unsuccessful due to the multiplex PCR techniques requiring further optimisation. Nonetheless, there is evidence of sand dune systems filtering bacteria (Price et al., 2013) and future investigations of site specific cases, where ecosystem services are being provided, could improve conservation priorities and efforts (Gross, 2006) within these vulnerable habitats.

6.5 Wider implications for management and conservation

6.5.1 Implications for a revised dune slack nitrogen critical load

Within a 3 month manipulated study (chapter 5) where groundwater nitrogen concentrations were maintained within static dune slack mesocosms with no groundwater flow; the mesocosms were

able to absorb/process as much as 3 kg N ha^{-1} yr^{-}, as well as the comparatively high background atmospheric deposition of 23 kg N ha^{-1} yr^{-} (Fig 5.2), and maintain a groundwater concentration of 0.2 mg l^{-1} of DIN. This study demonstrates that even at these low concentrations, nitrogen uptake (and loss) by the vegetation and soils are high, which implies that fluxes within a natural system, which are subject to these low groundwater concentrations, such as those measured at Aberffraw sand dune system (in chapter 2) can be quite high. Effectively, this suggests that such systems are probably subject to higher DIN groundwater concentrations than those measured; before the dune slack ecosystem, including the vegetation and soil microbe uptake, processes and accumulate the increased available nitrogen within the system. Nevertheless, this highlights how poorly we understand groundwater nitrogen fluxes and the consequences from groundwater nitrogen uptake into natural sand dune systems, however there is suggestion from chapter 2 of vegetation responses. This study therefore demonstrates the necessity to calculate input fluxes into natural sand dune systems impacted by eutrophication, to quantify a critical load for groundwater nitrogen contamination within dune slack habitats. These investigations will aid the construction of a revised nitrogen critical load, which will also consider groundwater nitrogen inputs into dune slack habitats.

6.5.2 Management

The management of dune slacks is common practice across much of Europe (Simpson, 1998, Grootjans et al., 2002, Leten et al., 2005). In many cases these systems are managed to avoid succession due to increased scrub invasions and lowered water table depths. These management techniques include mowing, grazing and sod-cutting, which help maintain species rich dune slacks. With regard to this study, however, we investigated the effects of groundwater nitrogen contamination along with lowering water tables on dune slack ecology and primarily concentrated on contamination inputs from agricultural practices. Within chapter 3 we demonstrate a combination of analytical techniques, to help identify potential nutrient contamination sources and pathways, which in turn, can inform appropriate management plans. Management plans appropriate to reduce nitrogen contamination inputs from agricultural practices include alterations in fertiliser application regimes which consider the time of year, weather conditions, soil type and crop uptake. Such planning before fertiliser application could reduce excess nitrogen losses into the groundwater, streams or as surface runoff from pastureland and in effect be more cost effective for farmers. The installation of buffer zones between sites and agricultural land, especially within areas where runoff events are identified, will reduce nitrogen inputs from runoff events and leaching as these zones enhance filtration of nutrients and decrease the rates of runoff (Patty et al., 1997).

6.6 Further research

Through the limitations of this study and lacking research, we highlight a number of studies and research questions, which may be of future beneficial value to the sand dune research community and wider wetland community. This study provided evidence of dune slack sensitivity to low nitrogen groundwater concentrations; however we are still unable to quantify at what annual fluxes of nitrogen inputs impacts occur on dune slack ecology. Such findings would allow for a revised nitrogen critical load value for dune slack habitats and would require a study, to quantify annual nitrogen fluxes within both, uncontaminated and contaminated sites with known impacts.

The efficacy of a combination of techniques to source the nature and pathways of nitrogen contamination was limited in differentiating faecal inputs (i.e. from cattle and sheep), however we were still able to differentiate inputs from on-site grazers and fertilisers. We therefore recommend the use of such techniques within contaminated wetland sites, fisheries zones and bathing waters globally to source, prevent and avoid human health implications from such contamination issues.

Lastly, even though we identified uptake of groundwater nitrogen within sand dune systems, we were limited in quantifying the specific uptake by microbes and vegetation. The use of labelled ^{15}N would allow for this and would provide a detailed nitrogen budget experiment. Furthermore chapters 4 and 5 only explored the impacts of a 10 cm lowered water table depth, rather than the predicted 100 cm drop by 2080 (Clarke and Ayutthaya, 2010). A study investigating lowered water tables at deeper depths, would allow for the appropriate research preparation, planning and conservation.

As a final point, we suggest the use of molecular qPCR techniques, to quantify the presence and diversity of the five key genes involved in nitrogen cycling *narG, napA, nirS, nirK,and nosZ* (Groffman et al., 2006). This investigation would demonstrate the microbial function of dune slacks and would clarify the implications from continued nitrogen contamination and lowered water tables on denitrification activity.

6.7 Final conclusions

The findings from the studies presented in this thesis have successfully identified that dune slack habitats are vulnerable from nitrogen groundwater contamination from concentrations as low as 0.2 mg l^{-1}, a concentration described as 'no cause for concern' on dune slack habitats (Davy et al., 2010). These concentrations were seen to impact dune slack soil chemistry and vegetation community structures, which include highly protected dune slack species. Our study also suggests that these

impacts are likely to be intensified by climate change or water abstraction, as lowered water tables were seen to decrease denitrification rates which subsequently increase soil nitrogen concentrations. The nitrogen uptake, processing and accumulation within sand dune systems increase when subject to groundwater contamination; consequently these systems provide a valuable ecosystem service by reducing water contamination whether it be for drinking water or bathing waters. These findings highlight the necessity to consider groundwater nutrient inputs in addition to atmospheric nitrogen inputs in wetland systems. For this to be implemented, fluxes of nitrogen into sand dune systems, with impacts from groundwater nutrient contamination, needs to be explored to construct new dune slack nitrogen critical loads.

6.8 References

ADEMA, E. B., GROOTJANS, A. P., PETERSEN, J. & GRIJPSTRA, J. 2002. Alternative stable states in a wet calcareous dune slack in The Netherlands. *Journal of Vegetation Science,* 13**,** 107-114.

ADEMA, E. B., VAN DE KOPPEL, J., MEIJER, H. A. & GROOTJANS, A. P. 2005. Enhanced nitrogen loss may explain alternative stable states in dune slack succession. *Oikos,* 109**,** 374-386.

BAKER, A. 2002. Fluorescence properties of some farm wastes: implications for water quality monitoring. *Water Research,* 36**,** 189-195.

BERENDSE, F., LAMMERTS, E. & OLFF, H. 1998. Soil organic matter accumulation and its implications for nitrogen mineralization and plant species composition during succession in coastal dune slacks. *Plant Ecology,* 137**,** 71-78.

BOBBINK, R. & HETTELINGH, J.-P. Review and revision of empirical critical loads and dose-response relationships. Proceedings of an expert workshop, Noordwijkerhout, 2010. 23-25.

BRAGAZZA, L., FREEMAN, C., JONES, T., RYDIN, H., LIMPENS, J., FENNER, N., ELLIS, T., GERDOL, R., HÁJEK, M. & HÁJEK, T. 2006. Atmospheric nitrogen deposition promotes carbon loss from peat bogs. *Proceedings of the National Academy of Sciences,* 103**,** 19386-19389.

CAMARGO, J. A. & ALONSO, A. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International,* 32**,** 831-849.

CLARKE, D. & AYUTTHAYA, S. S. N. 2010. Predicted effects of climate change, vegetation and tree cover on dune slack habitats at Ainsdale on the Sefton Coast, UK. *Journal of Coastal Conservation,* 14**,** 115-125.

CROWTHER, J., KAY, D. & WYER, M. D. 2002. Faecal-indicator concentrations in waters draining lowland pastoral catchments in the UK: relationships with land use and farming practices. *Water Res,* 36**,** 1725-1734.

CURRELI, A., WALLACE, H., FREEMAN, C., HOLLINGHAM, M., STRATFORD, C., JOHNSON, H. & JONES, L. 2013. Eco-hydrological requirements of dune slack vegetation and the implications of climate change. *Science of the Total Environment,* 443**,** 910-919.

DAVY, A., HISCOCK, K., JONES, M., LOW, R., ROBINS, N. & STRATFORD, C. 2010. Ecohydrological guidelines for wet dune habitats, phase 2. Protecting the plant communities and rare species of dune wetland systems. *Bristol, UK: Environment Agency*.

FIELD, K. G. & SAMADPOUR, M. 2007. Fecal source tracking, the indicator paradigm, and managing water quality. *Water Res,* 41**,** 3517-3538.

GROFFMAN, P. M., ALTABET, M. A., BÖHLKE, J., BUTTERBACH-BAHL, K., DAVID, M. B., FIRESTONE, M. K., GIBLIN, A. E., KANA, T. M., NIELSEN, L. P. & VOYTEK, M. A. 2006. Methods for measuring denitrification: diverse approaches to a difficult problem. *Ecological Applications,* 16**,** 2091-2122.

GROOTJANS, A., GEELEN, H., JANSEN, A. & LAMMERTS, E. 2002. Restoration of coastal dune slacks in the Netherlands. *Ecological Restoration of Aquatic and Semi-Aquatic Ecosystems in the Netherlands (NW Europe).* Springer.

GROSS, L. 2006. Assessing ecosystem services to identify conservation priorities. *PLoS Biology,* 4**,** e392.

JONES, L., NIZAM, M., REYNOLDS, B., BAREHAM, S. & OXLEY, E. 2013. Upwind impacts of ammonia from an intensive poultry unit. *Environmental Pollution,* 180**,** 221-228.

JONES, M. L. M., WALLACE, H. L., NORRIS, D., BRITTAIN, S. A., HARIA, S., JONES, R. E., RHIND, P. M., REYNOLDS, B. R. & EMMETT, B. A. 2004. Changes in vegetation and soil characteristics in coastal sand dunes along a gradient of atmospheric nitrogen deposition. *Plant Biology,* 6**,** 598-605.

LAMMERTS, E. & GROOTJANS, A. 1997. Nutrient deficiency in dune slack pioneer vegetation: a review. *Journal of Coastal Conservation,* 3**,** 87-94.

LAMMERTS, E., MAAS, C. & GROOTJANS, A. 2001. Groundwater variables and vegetation in dune slacks. *Ecological Engineering,* 17**,** 33-47.

LETEN, M., VAN NIEUWENHUYSE, H. & HERRIER, J.-L. 2005. Invasive scrub and trees in the coastal dunes of Flanders (Belgium): an overview of management goals, actions and results. *Proceedings 'dunes and estuaries*, 111-128.

MELTZER, J. & VAN DIJK, H. 1986. The effects of dissolved macro-nutrients on the herbaceous vegetation around dune pools. *Vegetatio,* 65, 53-61.

NELSON, E. J., KAREIVA, P., RUCKELSHAUS, M., ARKEMA, K., GELLER, G., GIRVETZ, E., GOODRICH, D., MATZEK, V., PINSKY, M., REID, W., SAUNDERS, M., SEMMENS, D. & TALLIS, H. 2013. Climate change's impact on key ecosystem services and the human well-being they support in the US. *Frontiers in Ecology and the Environment,* 11, 483-493.

PAERL, H. W. 1997. Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. *Limnology and Oceanography,* 42, 1154-1165.

PATTY, L., REAL, B. & GRIL, J. J. 1997. The use of grassed buffer strips to remove pesticides, nitrate and soluble phosphorus compounds from runoff water. *Pesticide Science,* 49, 243-251.

PEARCE, D. M. E. & CLYMO, R. S. 2001. Methane oxidation in a peatland core. *Global Biogeochemical Cycles,* 15, 709-720.

PLASSMANN, K., EDWARDS-JONES, G. & JONES, M. L. M. 2009. The effects of low levels of nitrogen deposition and grazing on dune grassland. *Science of the Total Environment,* 407, 1391-1404.

PRICE, W. D., BURCHELL, M. R., HUNT, W. F. & CHESCHEIR, G. M. 2013. Long-term study of dune infiltration systems to treat coastal stormwater runoff for fecal bacteria. *Ecological Engineering,* 52, 1-11.

RANWELL, D. 1959. Newborough Warren, Anglesey: I. The dune system and dune slack habitat. *The Journal of Ecology*, 571-601.

SEGERS, R. 1998. Methane production and methane consumption: a review of processes underlying wetland methane fluxes. *Biogeochemistry,* 41, 23-51.

SIMPSON, D. 1998. Bringing Back the Slacks: A review of the techniques used to restore and maintain the important dune slack communities at Ainsdale. *ENACT-PETERBOROUGH-,* 6, 9-11.

UKTAG 2014. United Kingdom Technical Advisory Group on the Water Framework Directive. Technical report on groundwater dependent terrestrial ecosystem (GWDTE) threshold values. V9; 23 June 2014.

VAN DEN BERG, L. J., TOMASSEN, H. B., ROELOFS, J. G. & BOBBINK, R. 2005. Effects of nitrogen enrichment on coastal dune grassland: a mesocosm study. *Environmental Pollution,* 138**,** 77-85.

WHALEN, S. 2005. Biogeochemistry of methane exchange between natural wetlands and the atmosphere. *Environmental Engineering Science,* 22**,** 73-94.

APPENDICES

APPENDIX I: **Published version of: Evidence for sensitivity of dune wetlands to groundwater nutrients**

Science of the Total Environment 490 (2014) 106–113

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Evidence for sensitivity of dune wetlands to groundwater nutrients

Jennifer Rhymes [a,*], Hilary Wallace [c], Nathalie Fenner [a], Laurence Jones [b]

[a] Bangor University, UK
[b] Centre for Ecology and Hydrology, UK
[c] Ecological Surveys (Bangor), UK

HIGHLIGHTS

- We studied a dune system surrounded by fertilised agricultural land.
- Groundwater nutrients affected vegetation and soils in dune slack wetlands.
- Change in vegetation and soil were observed at 0.2mg/L of DIN within groundwater.

ARTICLE INFO

Article history:
Received 17 December 2013
Received in revised form 7 April 2014
Accepted 8 April 2014
Available online xxxx

Editor: Eddy Y. Zeng

Keywords:
Dune slacks
Nitrogen
Groundwater
Contamination
Sand dunes
Ecohydrology

ABSTRACT

Dune slacks are seasonal wetlands, high in biodiversity, which experience considerable within-year and between-year variations in water-table. They are subject to many pressures including climate change, land use change and eutrophication. Despite their biological importance and the threats facing them, the hydrological and nutrient parameters that influence their soil properties and biodiversity are poorly understood and there have been no empirical studies to date testing for biological effects in dune systems resulting from groundwater nutrients at low concentrations. In this study we examined the impact of groundwater nutrients on water chemistry, soil chemistry and vegetation composition of dune slacks at three distance classes (0–150 m, 150–300 m, 300–450 m) away from known (off-site) nutrient sources at Aberffraw dunes in North Wales, whilst accounting for differences in water-table regime. Groundwater nitrate and dissolved inorganic nitrogen (DIN) and soil nitrate and nitrite all had significantly higher concentrations closest to the nutrient source. Multivariate analysis showed that although plant species composition within this site was primarily controlled by water table depth and water table fluctuation, nitrogen from groundwater also influenced species composition, independently of water table and soil development. A model containing all hydrological parameters explained 17% of the total species variance; an additional 7% was explained following the addition of NO_3 to this model. Areas exposed to elevated, but still relatively low, groundwater nutrient concentrations (mean 0.204 mg/L +/− 0.091 of DIN) had greater abundance of nitrophilous species and fewer basipholous species than in areas with lower concentrations. This shows that clear biological impact occurs below previously suggested DIN thresholds of 0.20–0.40 (mg/L).

1. Introduction

Sand dune systems have a global distribution (Martinez et al., 2004) and support a high biodiversity, including many threatened plant, insect and animal species (Rhind and Jones, 2009; Howe et al., 2010). They contain seasonal wetlands, known as dune slacks, which support a particularly diverse flora in Europe (Grootjans et al., 2004), including red list species such as the fen orchid *Liparis loeselii* and the liverwort *Petalophyllum ralfsii*.

Sand dune systems have undergone considerable change globally in the last century (Martinez et al., 2004). In temperate European dune systems these drivers include: changes in land use, crashing rabbit populations, climate change and eutrophication (Provoost et al., 2011; Jones et al., 2011; Beaumont et al., 2014). With regard to the latter; nutrients from atmospheric deposition have increased dramatically from their pre-industrial levels of 2–6 kg N ha^{-1} yr^{-1} (Fowler et al., 2004). As a consequence, the critical load defined for dune slacks, 10–15 kg N ha^{-1} yr^{-1} (Bobbink and Hettelingh, 2011), is exceeded across much of Europe. Whilst the effects of atmospheric deposition have received recent attention in dry dune habitats (Plassmann et al., 2009; Remke et al., 2009; Jones et al., 2013), relatively little attention has been given to the impact of other sources of nutrients in dune

* Corresponding author.
E-mail address: bspe2a@bangor.ac.uk (J. Rhymes).

http://dx.doi.org/10.1016/j.scitotenv.2014.04.029
0048-9697/

wetlands, indeed in wetlands in general, and the issue of groundwater or surface water-derived nutrients is not explicitly considered within atmospheric critical loads frameworks. In dune systems that are not isolated hydrologically from surrounding groundwater, there is the potential for nutrient inputs to these habitats from agricultural and other sources via groundwater to add to the nutrient load already received from atmospheric deposition. A collation of dune groundwater chemistry data (Davy et al., 2010) suggested that values >1 mg/L dissolved inorganic nitrogen (DIN) in dune groundwater indicated probable nutrient contamination of the groundwater within a site, whilst concentrations above 0.2 mg/L may also signify contamination. A global assessment of aquatic ecosystems concluded that concentrations above 0.5–1.0 mg/L of total nitrogen could lead to eutrophication (Camargo and Alonso, 2006). There have been studies in the Netherlands on impacts of highly eutrophic river water around drinking water infiltration ponds (Meltzer and van Dijk, 1986). However, there have been no empirical studies to date testing for biological effects in dune systems resulting from groundwater nutrients at low concentrations.

Species distribution within these ecosystems is governed primarily by water table depth, seasonal water table fluctuations and water chemistry (Curreli et al., 2012; Grootjans et al., 1996; Lammerts et al., 1992, 2001; Willis et al., 1959). Yet, there remains a major knowledge gap as to how groundwater nutrients may affect dune slack vegetation and at what concentrations (Jones et al., 2006). Studies of atmospheric nitrogen deposition impacts have been made in many habitats (e.g. Phoenix et al., 2012), with the potential for community shift in extreme cases such as conversion of heathlands into grasslands (Heil and Diemont, 1983). However, in dune slacks there is still relatively little empirical evidence of nutrient impacts either from atmospheric deposition or from other sources, especially at realistic N loads. One of the few studies, using high nutrient loads on dune vegetation at Braunton Burrows demonstrated that *Agrostis stolonifera* dominated a dune slack following surface additions of N and P (Willis, 1963).

Dune slack water tables tend to be at their highest in winter and fall in the summer months (Van Der Laan, 1979) as the water table is highly dependent on precipitation and evaporation. Water tables can also vary substantially from year to year (Ranwell, 1959; Stratford et al., 2013), causing periods of drought and flooding which affect the period in which the rooting zone is in contact with the water table. These fluctuations also play an important role in controlling nutrient composition within the soils. During periods of high water level, mineralisation of organic matter is reduced thus conserving the low nutrient status favoured by dune slack species (Berendse et al., 1998). Soil processes are important in regulating the impacts of N. Soil exchange sites may actively bind ammonium from the groundwater during periods of inundation, whilst denitrifying bacteria may release nitrogen back into the atmosphere (Myrold, 1998).

The aim of this investigation was to examine the impact of nutrients on dune groundwater chemistry, soil chemistry and botanical composition along gradients of nutrient input from known sources, and accounting for differences in water-table regime. We tested the following hypotheses: does nutrient contamination from off-site sources extend into the groundwater under the dune system? If nutrients are present in the groundwater, is there any evidence in the plant assemblages and soils of dune slacks that these nutrients are accessible to the vegetation in the dune slacks, and do they have an adverse ecological impact on the plant community composition?

2. Methods

2.1. Site description

Aberffraw dunes are located on the south west corner of the island of Anglesey in North Wales, UK (53°11′N, 4°27′W). The site extends for 1 km in width and 3 km inland (Fig. 1). A small lake, Llyn Coron bounds the north east edge of the system and feeds the river Afon Fraw, which

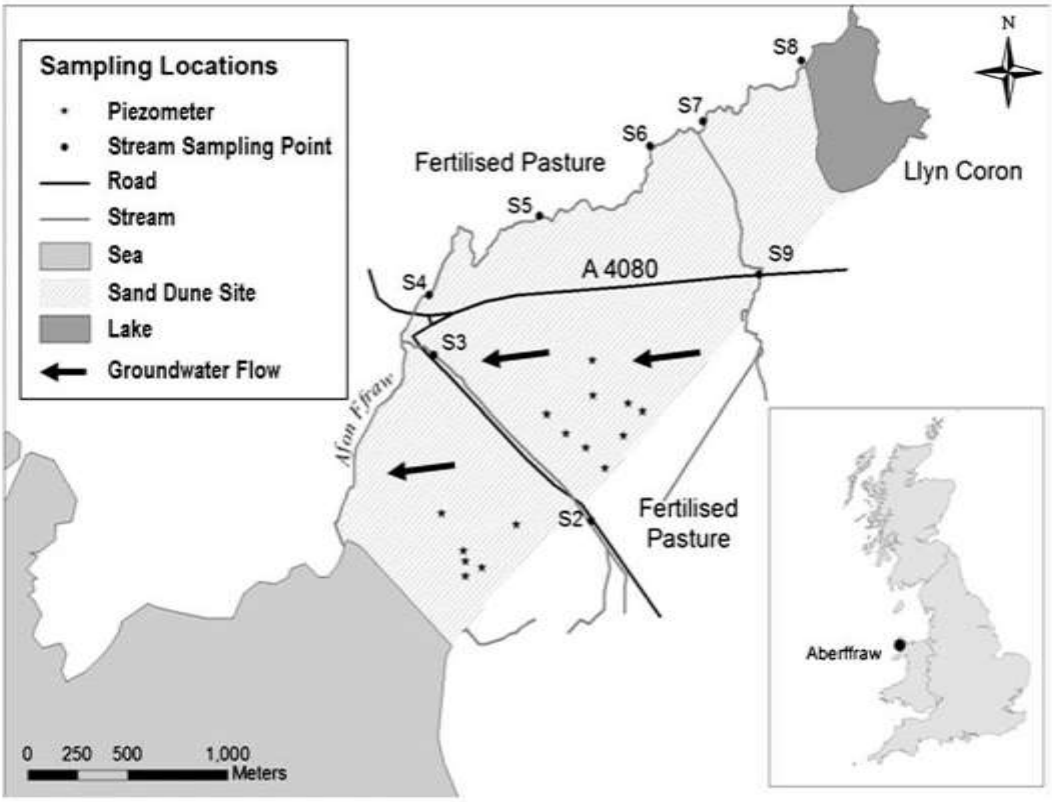


Fig. 1. Map of Aberffraw dune system, showing all piezometers and stream (S2–S9) sampling points. S1 (not shown) was an episodic stream and data were only collected from this sampling point for one month. Cross-hatched area represents designated site.
Redrawn from Ordnance Survey.

flows along the north-west edge of the dunes down to the sea. The site is in a low valley surrounded on all sides by agricultural land. The agricultural land is reseeded and fertilised pasture, used for sheep and cattle grazing, with feed stations on land immediately adjacent to the south-east dune site boundary. A number of streams and ditches draining this heavily fertilised agricultural area lead on to the site.

2.2. Groundwater flow direction

In a preliminary survey, elevation of the water table at each piezometer and at additional locations around the site was measured by auguring down to the water table and then referred to ground surface elevation measured using a Leica 1200 RTKGPS, with a vertical accuracy of ± 1 cm, and correcting for water table depth. Groundwater flow direction was estimated by contour analysis in ArcGIS v10.1.

2.3. Sampling design

A preliminary survey was carried out whereby water samples were collected by drilling down to the water table with an augur and sampling the groundwater with a hand pump. This established that there was a possible nitrate contamination gradient that extended into the site from the fertilised pastureland on the south-east site boundary. In order to quantify the possible effects of this contamination 15 piezometers, 2 m in depth with full-length slotted screens of 0.3 mm slots covered by mesh were installed. Installation was restricted to dune slack areas as this is where vegetation and rooting zones are in contact with the groundwater and where impacts are most likely to occur. The sampling strategy aimed at evaluating gradients in water chemistry within three distance classes from the south-east site boundary (0–150 m, 150–300 m and 300–450 m).

2.4. Hydrological monitoring and water chemistry sampling

Monthly manual measurements of groundwater levels were taken from 15 piezometers using a water level meter (Boart longyear), starting in March 2012 for a period of 12 months. Water samples were collected monthly from the top 10 cm of the water table at each piezometer. During periods of inundation, when water table was above ground level in certain slacks, samples of the standing water above the piezometer were collected. Water samples were also collected from streams entering or nearby the site (Fig. 1), which could potentially contribute to groundwater nutrients via seepage from the stream bed. Stream water samples were collected at the same time as groundwater, by dipping a clean collecting container into the surface flow. Samples were stored in darkness at 5 °C prior to chemical analysis. pH was recorded for each sample which was then filtered through 0.45 μm nylon syringe filter (Avonchem™). Dissolved inorganic anions (chloride, nitrite, nitrate, phosphate and sulphate) and cations (sodium, ammonium, potassium, calcium and magnesium) were then measured on an ion chromatograph (Metrohm, UK Ltd.). Detection limits for all anions and cations were 0.005 mg/L apart from nitrite (0.003 mg/L), nitrate (0.002 mg/L) and ammonium (0.001 mg/L). Dissolved inorganic nitrogen was calculated as the sum of NO_3–N, NO_2–N and NH_4–N.

2.5. Botanical survey

At each of the 15 piezometers vegetation was surveyed in three 1 m×1 m quadrats. The quadrats were placed at a 3 m distance from the piezometer and arranged on cardinal bearings (North, West and East). Species occurrence was recorded using visual estimates of % cover for all species of vascular plants, bryophytes and lichens. Nomenclature follows Stace (2010) for vascular plants and Hill et al. (1994) for bryophytes. Cover of bare ground and litter were also recorded. The location of each quadrat was recorded at its centre using a Leica 1200 RTKGPS. Mean UK-modified Ellenberg indicator values (Hill et al., 1999, 2007) were then calculated for each quadrat using species presence data.

2.6. Topographical resolution

Elevation of the ground surface at each piezometer and quadrat was measured using the Leica 1200 RTKGPS to 1 cm vertical resolution, which allowed groundwater levels for each quadrat to be calculated using their relative elevation difference from the nearest piezometer.

2.7. Soil sampling

At each quadrat a soil core (5 cm diameter, 15 cm depth) was collected and stored in darkness at 5 °C, prior to analysis. The thickness of the organic horizon was recorded and any vegetation and large roots were removed. The soil was then homogenised by hand and a sub-sample (10–15 g field moist soil) was weighed and dried at 105 °C and reweighed to measure moisture content. The samples were then re-heated in a furnace at 375 °C for 16 h and re-weighed to determine organic matter content through Loss on Ignition (Ball, 1964).

A sub-sample was prepared for chemical analysis using a water extraction of 10 g homogenised sample of fresh soil, mixed with 10 ml of ultra-high purity water (1:10 wt/vol) on a laboratory blender (Stomacher 80, Seward UK). pH was recorded using a calibrated pH electrode and electrical conductivity was measured using a conductivity meter (Primo 5, Hanna Instruments Ltd. UK). The remaining solution was centrifuged for 15 min at 5000 rpm and filtered through 0.45 μm nylon syringe filter (Avonchem™). Organic anions (chloride, nitrite, phosphate and sulphate) and cations (sodium, ammonium, potassium, calcium and magnesium) were then measured on the Metrohm ion chromatograph, detection limits described above.

2.8. Rooting depth

Soil pits > 30 cm wide and 1 m deep were dug at 5 m distance from six of the piezometers in order to measure rooting depth. Three of these were dug in slacks with a hydrological regime supporting wet slack vegetation communities and three in dry slack communities. On one clean vertical face in each soil pit, the number of visible roots in a 30 cm wide × 20 cm deep section were recorded at 4 depth bands below the surface (−20 to −40 cm, −40 to −60 cm, −60 to −80 cm and −80 to −100 cm). It was not possible to count visible roots in the main rooting zone (top layer 0 to −20 cm) due to the high abundance of roots.

2.9. Statistical analysis

Quadrats and piezometers were grouped into three classes based on their distance from the south-east site boundary (see Fig. 1) (0–150 m N = 15, 150–300 m N = 18, 300–450 m N = 12). Monthly groundwater (including inundation samples) and streamwater (N = 8) chemistry values and pH for each sampling point were averaged to give an annual mean, as preliminary analysis showed no seasonal differences in groundwater chemistry. Data from the three soil samples around each piezometer were also used to test for statistical differences among distance classes using analysis of variance (Minitab v16). Analysis of soil chemistry variables included annual maximum water table elevation as a co-variable. Data that proved not normally distributed (Kolmogorov–Smirnov Test) were transformed using a Johnson's transformation. Where transformation was not sufficient to achieve assumptions of normality – (Soils: phosphate, sodium and ammonium. Groundwater: sulphate and potassium) a non-parametric Kruskal–Wallis Test was carried out. Differences in

Table 1
Summary of annual mean water chemistry from piezometers and streams; values for each variable are expressed as mean ± standard error and brackets show minimum and maximum values. Values in bold show significant differences in groundwater chemistry among distance classes (see text).

Chemistry and pH	Groundwater	Streams
Chloride (mg/L)	68.717 ± 2.249 (16.113, 190.945)	44.141 ± 2.421 (22.707, 211.436)
Nitrite (mg/L)	0.008 ± 0.001 (0.003, 0.185)	0.042 ± 0.004 (0.005, 0.211)
Nitrate (mg/L)	**0.468 ± 0.112 (0.002, 16.706)**	10.945 ± 1.438 (0.003, 86.833)
Phosphate (mg/L)	0.006 ± 0.000 (0.005, 0.041)	0.058 ± 0.010 (0.005, 0.520)
Sulphate (mg/L)	16.887 ± 0.872 (1.088, 77.230)	14.376 ± 0.699 (1.550, 41.391)
Sodium (mg/L)	35.072 ± 1.093 (13.130, 92.294)	25.806 ± 0.997 (0.005, 123.956)
Ammonium (mg/L)	0.036 ± 0.005 (0.001, 0.585)	0.031 ± 0.005 (0.003, 0.221)
Potassium (mg/L)	1.857 ± 0.079 (0.005, 6.223)	4.398 ± 0.330 (0.005, 15.310)
Calcium (mg/L)	83.723 ± 1.336 (40.847, 187.050)	48.486 ± 1.657 (0.020, 94.846)
Magnesium (mg/L)	7.086 ± 0.167 (0.005, 14.720)	8.655 ± 0.184 (0.005, 21.234)
Dissolved inorganic N (mg/L)	**0.108 ± 0.002 (0.001, 3.829)**	2.485 ± 0.030 (0.002, 19.609)
pH	7.511 ± 0.021 (6.804, 8.473)	7.715 ± 0.027 (7.194, 8.620)

root abundance between wet and dry slack community soil pits were assessed using analysis of variance using Minitab v16.

Relationships between vegetation and measured soil and water variables were sought using multivariate analyses. An initial DCA of the 45 vegetation quadrats tested the length and strength of the first gradient whilst relationships between vegetation and environmental variables were explored through indirect gradient analysis using PCA. The significance of the relationships with environmental variables was tested singly and within models using Redundancy Analysis (RDA) Monte Carlo methods within CANOCO.

3. Results

3.1. Groundwater direction, groundwater and stream chemistry

The preliminary topographical and water level survey showed that the direction of groundwater flow is approximately westerly (Fig. 1). The summary data of annual piezometer water chemistry (Table 1) showed significant differences in annual mean groundwater nitrate concentrations of the piezometers in the three classes with those in the 0–150 m class (0.885 +/− 0.283 mg/L) being significantly greater than those in the 150–300 m (0.360 +/− 0.147 mg/L) or 300–450 m classes (0.092 +/− 0.046 mg/L). Significant difference was also found in annual mean groundwater dissolved inorganic nitrogen concentrations, with those in the 0–150 m class (0.204 +/− 0.091 mg/L) again being significantly greater than those in the 150–300 m (0.084 +/− 0.034 mg/L) or the 300–450 m classes (0.0224 +/− 0.011 mg/L). All other piezometer water chemistry variables showed no significant difference among classes. Nitrate and phosphate concentrations were an order of magnitude higher in the streams running through the site than in the dune groundwater, even in the class of piezometers nearest the south-east site boundary.

Nitrate concentrations at all stream sampling points (Fig. 2) do not exceed 20 mg/L apart from S2 which considerably exceeds this concentration. They show a slight seasonal trend with concentrations lower in summer than in winter. By contrast, the stream S2 which drains from the south-east boundary into the site shows a steep and rapid increase in nitrate concentration from mid-April that exceeds 50 mg/L for four months, peaking at 87 mg/L in June before rapidly declining from June to September to concentrations similar to other stream sampling points.

3.2. Soils

A summary of soil physico-chemistry parameters for the three classes of quadrats are shown in Table 2. Significantly higher soil nitrite concentrations occurred in the 0–150 m class (0.090 +/− 0.033 μg g^{-1} dry soil) compared with that of the 150–300 m class (0.034 +/− 0.010 μg g^{-1} dry soil) and the 300–450 m class (0.046 +/− 0.031 μg g^{-1} dry soil). Soil nitrate concentrations were also significantly greater

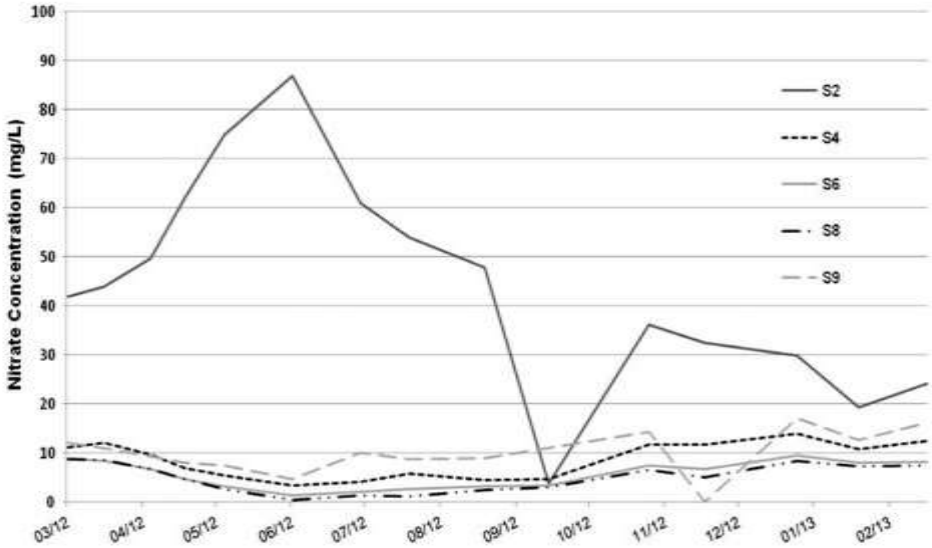


Fig. 2. Monthly nitrate concentrations (mg/L) from four representative stream sampling points and from divergent S2 (see Fig. 1) over a 12-month period. Samples from streams S3, S5, and S7 not shown for clarity, but were not significantly different from S4–S9 shown here.

Table 2
Summary of soil physico-chemistry parameters for categorised quadrats located 0–150 m (n = 15), 150–300 m (n = 18) and 300–450 m away from the south-east site boundary (n = 12). Values for each variable are expressed as mean ± standard error; brackets show minimum and maximum values for each class. Significant differences among distance classes shown in bold; values denoted by the same letter not significantly different from each other.

Variable		Distance from fence		
		0–150	150–300	300–450
		n = 15	n = 18	n = 12
Soil chemistry (μg g^{-1} dry soil)	Chloride	4.105 ± 0.243 (2.307, 5.597)	3.742 ± 0.334 (2.318, 7.950)	4.532 ± 0.573 (2.337, 10.006)
	Nitrite	**0.090 ± 0.033^{A} (0.020, 0.519)**	**0.034 ± 0.010^{B} (0.005, 0.159)**	**0.046 ± 0.031^{B} (0.005, 0.390)**
	Nitrate	**1.967 ± 0.515^{A} (0.397, 7.838)**	**0.612 ± 0.155^{B} (0.013, 2.485)**	**1.087 ± 0.730^{B} (0.018, 9.051)**
	Phosphate	0.018 ± 0.005 (0.011, 0.081)	0.013 ± 0.001 (0.012, 0.025)	0.013 ± 0.000 (0.011, 0.014)
	Sulphate	3.630 ± 0.229 (2.176, 5.420)	3.713 ± 0.158 (2.671, 4.868)	3.822 ± 0.441 (2.100, 7.302)
	Sodium	2.846 ± 0.660 (0.011, 6.886)	3.419 ± 0.733 (0.012, 7.910)	3.279 ± 0.792 (0.012, 7.295)
	Ammonium	0.350 ± 0.061 (0.012, 0.490)	0.367 ± 0.057 (0.012, 2.423)	0.622 ± 0.210 (0.013, 0.849)
	Potassium	3.813 ± 0.610 (1.927, 8.228)	4.414 ± 0.479 (1.214, 35.954)	6.174 ± 2.460 (0.631, 10.213)
	Calcium	29.244 ± 3.754 (10.790, 41.567)	28.636 ± 3.185 (0.045, 39.389)	28.868 ± 2.396 (0.034, 52.584)
	Magnesium	1.709 ± 0.639 (0.639, 2.339)	2.165 ± 0.162 (0.412, 3.604)	2.169 ± 0.198 (1.669, 3.481)
	Dissolved Inorganic N	0.744 ± 0.152 (0.041, 0.753)	0.434 ± 0.067 (0.060, 4.047)	0.743 ± 0.321 (0.324, 2.588)
Soil characteristics	pH	7.690 ± 0.147 (6.480, 8.370)	7.990 ± 0.104 (6.952, 8.556)	8.054 ± 0.144 (6.703, 8.640)
	Electrical conductivity (mS/cm)	28.867 ± 3.216 (12.000, 50.000)	31.167 ± 2.472 (13.000, 48.000)	30.667 ± 3.438 (11.000, 57.000)
	LOI (%)	5.020 ± 0.421 (1.760, 7.618)	4.674 ± 0.496 (0.464, 9.189)	4.728 ± 0.597 (2.588, 8.870)
	Observed organic matter (cm)	8.286 ± 0.947 (2.000, 15.000)	9.941 ± 0.860 (3.000, 15.000)	10.250 ± 1.008 (5.000, 15.000)

within the 0–150 m class (1.967 +/− 0.515 μg g^{-1} dry soil) compared with that of both the 150–300 m (0.612 +/− 0.155 μg g^{-1} dry soil) and 300–450 m class (1.087 +/− 0.730 μg g^{-1} dry soil). No significance among classes was found for soil dissolved inorganic nitrogen and for all other variables.

3.3. Vegetation

Fig. 3 shows water table variation in relation to rooting depth for two hydrographs typical of a wet and a dry slack community, corresponding to UK National Vegetation Classification SD14d (*Salix repens–Campyllium stellatum* dune slack, *Festuca rubra* subcommunity) and SD15b (*Salix repens–Calliergon cuspidatum* dune slack, *Equisetum variegatum* subcommunity) respectively (Rodwell, 2000). Both wet and dry slack communities reveal an asymmetric seasonal pattern whereby the water depth drops steadily over the summer period and increases rapidly in early winter (Fig. 3a). The summary data for hydrological parameters (Fig. 3b) shows the range of fluctuation in water depth with the average minimum at −105 cm and maximum at 48 cm (i.e. above ground surface), with one piezometer as high as 193 cm. The dry slack community has a lower water depth than that of a wet slack all year around,

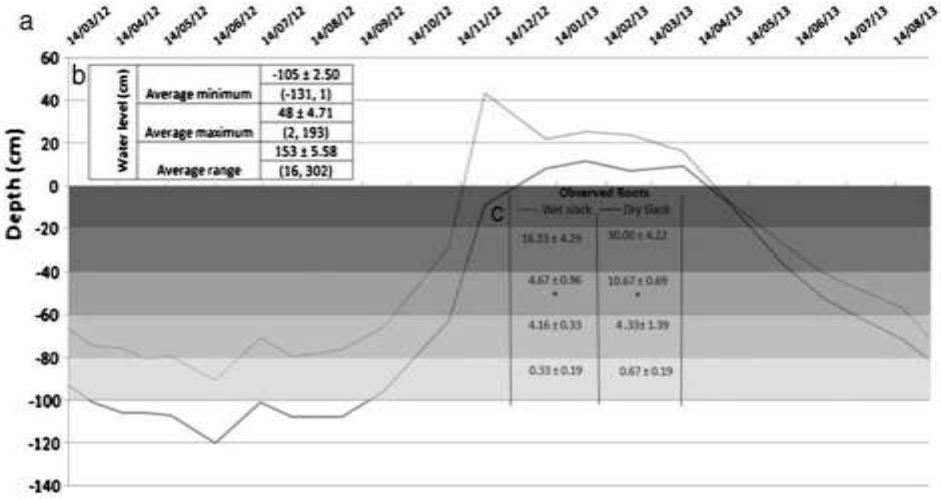


Fig. 3. a) Annual hydrographs of two piezometers from a wet and a dry slack. b) Annual average of minimum, maximum and range water level data from 13 piezometers expressed as mean ± SE, brackets show minimum and maximum values. c) Visible roots observed at 4 rooting depth zones (−20 to −40 cm, −40 to −60 cm, −60 to −80 cm and −80 to −100 cm) expressed as mean ± SE. Asterisks denote a significant difference between root abundance in dry slacks SD 14d and wet slacks SD 15b.

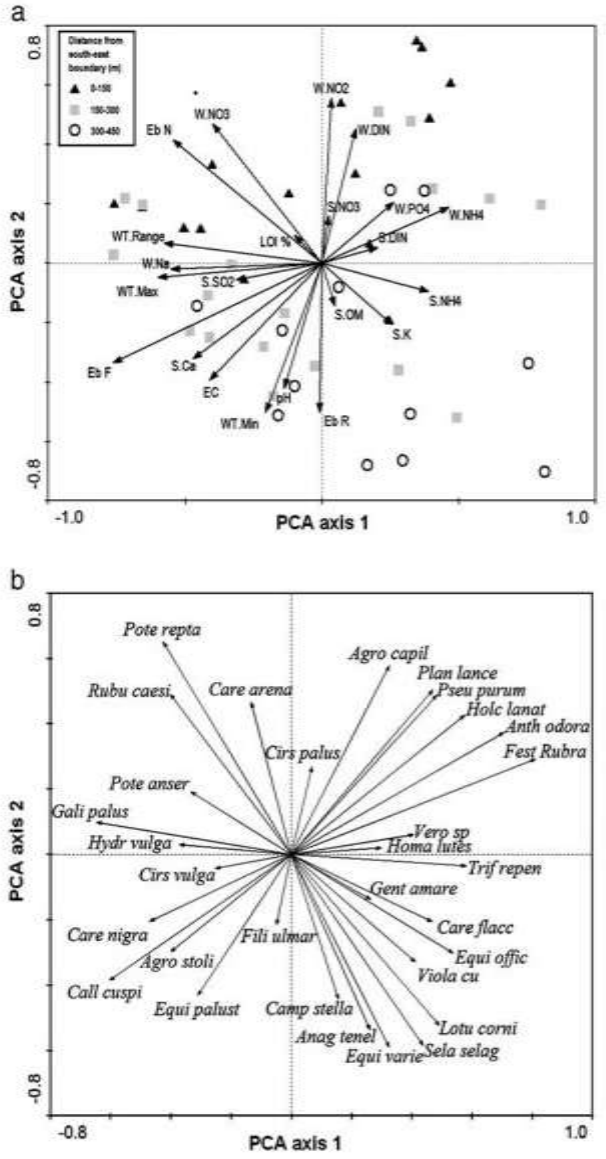


Fig. 4. PCA analysis: (a) the distribution of environmental variables with PCA scores, quadrats coded by distance to south-east site boundary. (b) The distribution of species with PCA scores. Only environmental variables and species with axis scores >0.2 are shown for clarity (except LOI). See Tables 2 and 3 for full list of variables. Prefixes denote the following: S — Soil; W — groundwater and WT — water table and pH — soil pH.

with the greatest differentiation occurring in the drier months of summer (Fig. 3a). Roots were significantly more abundant at depths −40 to −60 cm in dry communities than in wet communities (Fig. 3c), there was no significant difference at all other depths. In the wet slack vegetation community, the main rooting zone (0 to −40 cm) is in contact with the water table for approximately 8 months. In the dry slack vegetation community, the main rooting zone (0 to −60 cm) is also in contact with the water table for a similar duration, suggesting rooting depth is constrained by water levels.

The PCA plot shows the distribution of the 45 quadrats, coded by their distance to the south-east site boundary, with environmental variables overlain to aid interpretation (Fig. 4). The overlain environmental variables suggest that axis 1 (Fig. 4a) relates to a hydrological gradient in which annual maximum water level, water level range and Ellenberg F were negatively associated with the axis. i.e. high water tables were found to the left of the diagram, corresponding to low scores on axis 1. The low axis 1 scores (Fig. 4b) were occupied by species tolerant of wet soils *Galium palustre*, *Hydrocotyle vulgaris* and *Carex nigra* whereas the highest axis 1 scores were occupied by drier species such as *Lotus corniculatus* and *Trifolium repens*. Axis 2 (Fig. 4a) related to a combined soil development/nutrient axis where groundwater NO_2 concentrations, soil NO_3 concentrations and Ellenberg N were positively linked with axis 2, and soil pH and Ellenberg R were negatively associated with the axis. The overlain species data reinforce this pattern, with low axis 2 scores (Fig. 4b) occupied by species with higher base status demand such as *C. stellatum* and *E. variegatum* whereas high scores were revealed by higher fertility species e.g. *Rubus caesius* and *Potentilla reptans*. There was no clear separation of quadrats relative to distance to south-east site boundary on Axis 1, however axis 2 (Fig. 4b) segregated the 0–150 m class from the 300–450 m class, with quadrats in the 0–150 m class located higher on axis 2.

Using a Monte Carlo permutation test the model containing all variables was highly significant ($p < 0.001$), where the first four axes explained 51.8% of the species–environment relationships and explained 45.9% of the total species variance. Most variables tested singly were significant at 0.001 level (Table 3). A model containing all hydrological parameters explained 17% of the total species variance, showing as expected a degree of co-correlation between hydrological variables. When tested singly, NO_3 explained more of the total species variance than any of the individual hydrological variables. When the influence of all hydrological variables was accounted for in a combined model, adding NO_3 explained an additional 7% of species variance. This shows that species variation due to groundwater NO_3 was largely independent of that due to hydrology, and that NO_3 was significantly affecting plant community composition.

4. Discussion

This study has shown that there is a nutrient contamination gradient that extends from the south-east site boundary into the site which is significantly affecting groundwater nitrate and dissolved inorganic N concentrations, soil nitrate and nitrite concentrations and vegetation composition.

Results suggest that the contamination is sourced from the south-east fertilised pasture land and is likely to be due to fertiliser application. Concentrations of nitrate samples from stream S2, which flows onto the site, exceed the 50 mg/L nitrate vulnerable zones designation threshold (Environment Agency, 2012) and the 50 mg/L World Health Organisation's guideline value for drinking water. Contamination is not likely to be due to manure within the site, or from the adjoining pasture land as ammonium and phosphate concentrations are relatively low within the streams, groundwater and soils. In sandy soils with low water holding capacity it is probable that nitrate is rapidly leached post fertiliser application (Skiba and Wainwright, 1984), particularly after heavy periods of rainfall and in turn is contaminating the groundwater. The sandy nature of the pasture land at Aberffraw allows groundwater to carry pollutants in a westerly direction into the site but the flow rate is unknown, although a study carried out at a nearby site, Newborough Warren, determined that groundwater flows at a speed of 39.6 m/year (Betson et al., 2002). This suggests at least 3 years of contamination as NO_3 concentrations are elevated at up to 150 m into the site. Although knowledge of the local land management history suggests that the adjacent farmland has been intensively managed for several decades and such nutrient concentrations are unlikely to be a recent phenomenon. Since nitrate concentrations determined from stream S2 are much greater than those determined in the groundwater, this suggests that the spatial extent of contamination could represent a number of possibilities: 1) an equilibrium caused by physical dilution and mixing with uncontaminated rainwater that infiltrates through the sand or 2) a result of processing and denitrifying N within the sandy body, or 3) a combination of both processes. Further work is required to assess to what extent dilution and denitrification play a significant role in reducing NO_3 concentrations in this aquifer.

Table 3
Environmental variables illustrating percentage of total species variation explained within RDA and significance, when tested singly.

	Variables	Variance (%)	Significance
Hydrological variables	Annual maximum water level (m)	8.80%	***
	Annual minimum water level (m)	7.30%	***
	Annual Range (m)	8.40%	***
Soil variables	S.Ca (μg g^{-1} dry soil)	7.30%	***
	S.NH_4 (μg g^{-1} dry soil)	5.90%	**
	S.Mg (μg g^{-1} dry soil)	5.80%	**
	S.pH	5.30%	*
	S.DIN (μg g^{-1} dry soil)	4.00%	***
	EC (mS/cm^{-1})	7.60%	*
	Soil moisture (%)	4.00%	*
	LOI (%)	2.20%	***
Water chemistry	W.NO_3 (mg/L)	9.00%	***
	W.Na (mg/L)	8.20%	***
	W.NO_2 (mg/L)	7.90%	***
	W.NH_4 (mg/L)	7.20%	***
	W.Cl (mg/L)	6.80%	***
	W.DIN (mg/l)	6.10%	**
	W.Br (mg/L)	5.10%	**
	W.PO_4 (mg/L)	4.80%	*
	W.K (mg/L)	4.60%	*
	W.SO_2 (mg/L)	4.10%	*
	W.Ca (mg/L)	4.00%	*
	W.Mg (mg/L)	4.00%	***
Ellenberg indicators	Eb F	13.60%	***
	Eb N	9.80%	***
	Eb R	6.20%	***
Combined models	Hydrological parameters (min, max + range)	17.0%	***
	Hydrological parameters and groundwater nitrate (min, max, range, W.NO_3)	24.0%	

* Significant at 0.05 level.
** Significant at 0.01 level.
*** Significant at 0.001 level.

The observed nitrate and nitrite soil gradient is likely to be due to uptake from the groundwater during the winter and spring months, when water tables are at their highest and plant roots are in direct contact with the groundwater or capillary fringe. This allows for possible nutrient uptake by the plants and subsequently the nutrients return to the soil surface via litter fall (Berendse et al., 1998), and direct binding of ammonium by the soil. As farming on this pastureland has been carried out for decades it is likely that the nutrient gradient has accumulated over time, but has not yet lead to significantly increased organic matter accumulation. This could be due to microbial processes maintaining low nutrient levels, as denitrification has been found to significantly increase with NO_3 availability (Merrill and Zak, 1992). It is also likely that in areas of contamination a shift in the microbial community composition has occurred, which supports higher levels of microbial activity (Peacock et al., 2001) and therefore maintaining low organic matter build up. Although denitrification rates can also be limited by other nutrients such as available carbon (Weier et al., 1993).

Assessment of the rooting zones has determined that the water table is likely to be a major factor controlling the rooting depth and as a result the main rooting zones within wet slacks are found in the shallower 0 to −40 cm zone compared with those within dry slacks in the deeper −0 to −60 cm zone. With differing water table regimes in both communities and the effects of capillary reaction, which carries substantial amounts of water 45 cm above it (Ranwell, 1959), both wet and dry slacks main rooting zones are exposed to groundwater for similar periods of the year and therefore are equally vulnerable to groundwater contamination.

Although the main determinant of species composition was water table depth and water table fluctuation, in broad agreement with the literature (e.g. Lammerts et al., 2001), RDA analysis showed that nitrogen was strongly influencing species composition independently of water table and soil development. The results suggest that with increasing availability of N basiphilous species have decreased, whilst species with higher nutrient status have increased.

If nitrogen pollution within this system continues it is likely that over time the slacks will become more eutrophic, resulting in greater productivity, more rapid soil development, increase in succession rate and loss of species (Jones et al., 2008). Other issues of concern are the projected changes in hydrological regimes, due to climate change, from wet dune slack regimes to dry grassland regimes (Curreli et al., 2012). This is likely to increase the mineralisation of organic matter, such that the desired low nitrogen and phosphorus conditions are not preserved (Lammerts and Grootjans, 1997), which will further exacerbate the eutrophication issue. This study is the first evidence that shows biological impact caused by DIN groundwater concentrations below 0.2 mg N/L within dune wetlands, which is below threshold concentrations described by Davy et al. (2010) and Camargo and Alonso (2006).

5. Implications for management

Sandy soils contain very little organic matter or cation exchange sites and therefore have low potential to store nitrogen in the soil, and leach nitrate readily (Rowell, 1994). As a result, it is more cost effective for farmers operating on sandy soils to only apply enough nitrogen that can be directly utilised by the crop. Site specific measures to reduce excess N leaving the site could include a new fertiliser application regime whereby less fertiliser is applied in more frequent doses which will reduce the loss of nitrate through leaching, and installation of fenced buffer zones along pastureland edges and ditches to enhance filtration of nutrients and decrease the rates of runoff (Patty et al., 1997).

6. Conclusions

Aberffraw dune system is exposed to a nutrient gradient in groundwater which is likely to be caused by farming practices on surrounding pastureland. Plant species composition of dune slack wetlands within this site is primarily controlled by water table depth and water table fluctuation. However nitrogen from groundwater is influencing species composition independently of water table and soil development, with evidence of an increase in more eutrophic species and a decrease in basiphilous species in affected areas. Whilst there is increasing evidence of N impacts in dry dune habitats (e.g. Jones et al., 2004, 2013; van den Berg et al., 2005; Kooijman, 2004), this is the first field-based evidence for impacts of N in dune slacks at relatively low groundwater nutrient concentrations. This study highlights two key findings: impacts have been observed at very low nutrient concentrations of around 0.2 mg/L DIN, reinforcing potential impacts on aquatic systems at low levels of N (Camargo and Alonso, 2006). Further, it shows that groundwater nutrient inputs need to be considered in addition to atmospheric N inputs in wetland systems. However, additional work is needed to determine the fluxes of N entering the site, in order to match the critical load approach. Experimental approaches to investigate groundwater nutrient impacts would also be useful, but technically difficult to implement.

Acknowledgements

This project has been made possible by the EU's Convergence European Social Fund through the Welsh Assembly Government ESF Programme 2007–2013 project number c80300 and co-funded by Ecological Surveys (Bangor) and the Natural Environment Research Council. We thank all technicians in Wolfson Carbon Capture Lab and Centre for Ecology and Hydrology Labs, for support with water chemistry and soil analysis. We also thank field assistant Aimée Toft.

References

Ball DF. Soils and land-use of the district around Bangor and Beaumaris. Mem. Soil surv. Gt. Britain. London: H.M.S.O.; 1964.

Beaumont NJ, Jones L, Garbutt A, Hansom JD, Toberman M. The value of carbon sequestration and storage in UK coastal habitats. Estuar Coast Shelf Sci 2014; 137:32–40. http://dx.doi.org/10.1016/j.ecss.2013.11.022.

Berendse F, Lammerts EJ, Olff H. Soil organic matter accumulation and its implications for nitrogen mineralisation and plant species composition during succession in coastal dune slacks. Plant Ecol 1998;137:71–8.

Betson M, Connell M, Bristow C. Groundwater modelling of Newborough Warren: a report for the Countryside Council for Wales, Volume 5, Hydrogeology. The impact of forestry on coastal geomorphology at Newborough Warren/Ynys Llanddwyn NNR, SSSI, pSAC; 2002.

Bobbink R, Hettelingh JP, editors. Review and revision of empirical critical loads and dose–response relationships. Proceedings of an expert workshop, Noordwijkerhout, 23–25 June 2010RIVM, NL: Coordination Centre for Effects; 2011.

Camargo JA, Alonso A. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environ Int 2006;32:831–49.

Curreli A, Wallace H, Freeman C, Hollingham M, Stratford C, Johnson H, et al. Eco-hydrological requirements of dune slack vegetation and the implications of climate change. Sci Total Environ 2012;443:910–9.

Davy AJ, Hiscock KM, Jones MLM, Low R, Robins NS, Stratford C. Ecohydrological guidelines for wet dune habitats, phase 2. Protecting the plant communities and rare species of dune wetland systems. Bristol, UK: Environment Agency; 2010.

Environment Agency. Method statement for nitrate vulnerable zone review. Surface waters environment agency report to Defra and Welsh Government — supporting paper for the implementation of the nitrates directive 2013–2016. Bristol: Environment Agency; 2012. [http://archive.defra.gov.uk/environment/quality/water/waterquality/diffuse/nitrate/documents/nvz-eutrophic-method.pdf].

Fowler D, O'Donoghue M, Muller JBA, Smith RI, Dragosits U, Skiba U, et al. A chronology of nitrogen deposition in the UK between 1900 and 2000. Water Air Soil Pollut 2004;4:9–23.

Grootjans AP, Sival FP, Stuyfzand PJ. Hydro-geochemical analysis of a degraded dune slack. Vegetation 1996;126:27–38.

Grootjans AP, Adema EB, Bekker RM, Lammerts EJ. Why coastal dune slacks sustain a high biodiversity. In: Martinez ML, Psuty NP, editors. Coastal dunes: ecology and conservation. Ecological Studies Berlin: Springer; 2004. p. 85–101.

Heil GW, Diemont WH. Raised nutrient levels change heathland into grassland. Vegetation 1983;53:113–20.

Hill MO, Preston CD, Smith AJE. Atlas of the bryophytes of Britain and Ireland. Colchester: Harley Books; 1994.

Hill MO, Mountford J, Roy D, Bunce R. Ellenber's indicator values for British plants: ECOFACT volume 2 technical annex HMSO; 1999.

Hill MO, Preston CD, Bosanquet SDS, Roy DB. Attributes of British and Irish Mosses, Liverworts and Hornworts with information on native status, size, life forum, life history, geography and habitat. BRYOATT; 2007 [88 pp.].

Howe MA, Knight GT, Clee C. The importance of coastal dunes for terrestrial invertebrates in Wales and the UK, with particular reference to aculeate Hymenoptera (bees, wasps & ants). J Coast Conserv 2010;14(2):91–102.

Jones MLM, Wallace HL, Norris D, Brittain SA, Haria S, Jones RE, et al. Changes in vegetation and soil characteristics in coastal sand dunes along a gradient of atmospheric nitrogen deposition. Plant Biol 2004;6:598–605.

Jones MLM, Reynolds B, Brittain SA, Norris DA, Rhind PM, Jones RE. Complex hydrological controls on wet dune slacks: the importance of local variability. Sci Total Environ 2006;372:266–77.

Jones MLM, Sowerby A, Williams DL, Jones RE. Factors controlling soil development in sand dunes: evidence from a coastal dune soil chronosequence. Plant Soil 2008; 307(1–2):219–34.

Jones L, Angus S, Cooper A, Doody P, Everard M, Garbutt A, et al. Coastal Margins (chapter 11). In: UK National Ecosystem Assessment, editor. Understanding nature's value to society Cambridge: Technical Report. UNEP-WCMCP.411-57; 2011.

Jones L, Nizam MS, Reynolds B, Oxley ERB, Bareham S. Upwind impacts of ammonia from an intensive poultry unit. Environ Pollut 2013;180:221–8.

Kooijman AM. Environmental problems and restoration in coastal dunes in the Netherlands. In: Martínez ML, et al, editors. Coastal dunes: ecology and conservation. Ecological Studies Berlin: Springer; 2004. p. 243–58.

Lammerts EJ, Grootjans AP. Nutrient deficiency in dune slack pioneer vegetation: a review. J Coast Conserv 1997;3:87–94.

Lammerts EJ, Sival FP, Grootjans AP, Esslink H. Hydrological conditions and soil buffering processes controlling the occurrence of dune slack species on the Dutch Wadden Sea islands. Coastal dunes: geomorphology, ecology and management for conservation; 1992. p. 265–72.

Lammerts EJ, Mass C, Grootjans AP. Groundwater variables and vegetation in dune slacks. Ecol Eng 2001;17:33–47.

Martinez ML, Psuty NP, Lubke RA. A perspective on coastal dunes. In: Martínez ML, et al, editors. Coastal dunes: ecology and conservation, 171. Ecological Studies; 2004. p. 3–10.

Meltzer JA, van Dijk HWJ. The effects of dissolved macro-nutrients on the herbaceous vegetation around dune pools. Vegetation 1986;65:53–61.

Merrill AG, Zak DR. Factors controlling denitrification rates in upland and swamp forests. Can J For Res 1992;22:1597–604.

Myrold DD. Transformations of nitrogen. In: Sylvia DM, Fuhrmann JJ, Hartel PG, Zuberer DA, editors. Principles and applications of soil microbiology. Upper Saddle River, NJ, USA: Prentice Hall; 1998. p. 259–94.

Patty L, Benoît R, Gril JJ. The use of grassed buffer strips to remove pesticides, nitrate and soluble phosphorus compounds from runoff water. Pestic Sci 1997;49: 243–51.

Peacock AD, Mullen MD, Ringelberg DB, Tyler DD, Hedrick DB, Gale PM, et al. Soil microbial community responses to dairy manure or ammonium nitrate applications. Soil Biol Biochem 2001;33:1011–9.

Phoenix GK, Emmett BA, Britton AJ, Caporn SJM, Dise NB, Helliwell R, et al. Impacts of atmospheric nitrogen deposition: responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments. Global Change Biol 2012;18(4):1197–215.

Plassmann K, Edwards-Jones G, Jones MLM. The effects of low levels of nitrogen deposition and grazing on dune grassland. Sci Total Environ 2009;407:1391–404.

Provoost S, Jones MLM, Edmondson SE. Changes in landscape and vegetation of coastal dunes in northwest Europe: a review. J Coast Conserv 2011;15:207–26.

Ranwell D. Newborough-Warren, Anglesey. 1. The dune system and dune slack habitat. J Ecol 1959;47:571–601.

Remke E, Brouwer E, Kooijman AM, Blindow I, Esselink H, Roelefs JGM. Even low to medium nitrogen deposition impacts vegetation of dry coastal dunes around the Baltic Sea. Environ Pollut 2009;157:792–800.

Rhind PM, Jones R. A framework for the management of sand dune systems in Wales. J Coast Conserv 2009;13:15–23.

Rodwell JS. British plant communities. Maritime communities and vegetation of open habitats, vol. 5. Cambridge University Press; 2000.

Rowell DL. Soil science: methods and applications. Longman Scientific & Technical. Harlow, Essex, UK: Longman Group UK Ltd.; 1994.

Skiba U, Wainwright M. Nitrogen transformation in coastal sands and dune soils. J Arid Environ 1984;7:1–8.

Stace C. New flora of the British Isles. 3rd ed. Cambridge, UK: Cambridge University Press; 2010.

Stratford C, Robins NS, Clarke D, Jones L, Weaver G. Hydroecology of fragile coastal dune slacks on the west coast of England and Wales. Ecohydrology 2013;6(1): 162–71.

Van Den Berg LJL, Tomassen HBM, Roelofs JGM, Bobbink R. Effects of nitrogen enrichment on coastal dune grassland: a mesocosm study. Environ Pollut 2005;138:77–85.

Van Der Laan D. Spatial and temporal variation in the vegetation of dune slacks in relation to the ground water regime. Vegetation 1979;39:43–52.

Weier KL, Doran JW, Power JF, Walters DT. Denitrification and the dinitrogen nitrous-oxide ratio as affected by soil-water, available carbon and nitrate. Soil Sci Soc Am J 1993;57:66–72.

Willis AJ. Braunton Burrows: the effects on the vegetation of the addition of mineral nutrients to the dune soils. J Ecol 1963;51:353–74.

Willis AJ, Folkes BF, Hopesimpson JF, Yemm EW. Braunton burrows — the dune system and its vegetation, part 1. J Ecol 1959;47:1–24.

APPENDIX II: **Published version of: Using chemical, microbial and fluorescence techniques to understand contaminant sources and pathways to wetlands in a conservation site.**

Science of the Total Environment 511 (2015) 703–710

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Using chemical, microbial and fluorescence techniques to understand contaminant sources and pathways to wetlands in a conservation site

J. Rhymes [a,*], L. Jones [b], D.J. Lapworth [c], D. White [c], N. Fenner [a], J.E. McDonald [a], T.L. Perkins [a]

[a] Bangor University, UK
[b] Centre for Ecology and Hydrology, UK
[c] British Geological Survey, UK

HIGHLIGHTS

- We used chemistry, fluorescence and bacterial counts to study multiple pathways of contamination to a wetland site.
- Contamination sources are primarily fertilizers, causing exceedance of nutrient thresholds in groundwater within the site.
- Contamination pathways into the site include groundwater, surface runoff, and streams, with minor input from on-site grazers.
- The site attenuates nutrient and bacterial concentrations providing an ecosystem service, but with adverse biological effects.

ARTICLE INFO

Article history:
Received 5 November 2014
Received in revised form 23 December 2014
Accepted 24 December 2014
Available online xxxx

Editor: D. Barcelo

Keywords:
Faecal indicator bacteria
Dissolved organic matter fluorescence
Water chemistry
Groundwater
Sand dunes
Dune slacks
Ecosystem service

ABSTRACT

Nutrients and faecal contaminants can enter wetland systems in a number of ways, with both biological and potentially human-health implications. In this study we used a combination of inorganic chemistry, dissolved organic matter (DOM) fluorescence and *Escherichia coli* and total coliform (TC) count techniques to study the sources and multiple pathways of contamination affecting a designated sand dune site of international conservation importance, surrounded by agricultural land. Analysis of stream samples, groundwater and dune slack wetlands revealed multiple input pathways. These included riverbank seepage, runoff events and percolation of nutrients from adjacent pasture into the groundwater, as well as some on-site sources. The combined techniques showed that off-site nutrient inputs into the sand dune system were primarily from fertilisers, revealed by high nitrate concentrations, and relatively low tryptophan-like fulvic-like ratios <0.4 Raman units (R.U.). The *E. coli* and TC counts recorded across the site confirm a relatively minor source of bacterial and nutrient inputs from on-site grazers. Attenuation of the nutrient concentrations in streams, in groundwater and in run-off inputs occurs within the site, restoring healthier groundwater nutrient concentrations showing that contaminant filtration by the sand dunes provides a valuable ecosystem service. However, previous studies show that this input of nutrients has a clear adverse ecological impact.

1. Introduction

The global availability and mobility of nitrogen have increased rapidly over the past five decades (Galloway and Cowling, 2002) and the damaging impacts it has on freshwater ecosystems are widely documented (Camargo and Alonso, 2006). Aquatic systems are extremely sensitive to nitrogen and are threatened by atmospheric deposition inputs (Fowler et al., 2005) as well as point sources and diffuse sources which can enter aquatic systems via numerous pathways such as through runoff, streams and groundwater. Tracing sources of aquatic pollution is therefore often problematic (Withers et al., 2009).

Within rural areas river water quality (Hooda et al., 2000) and groundwater quality (Oakes et al., 1981) are primarily impacted by agricultural diffuse pollution (Novotny, 1999). Atmospheric nutrients have been demonstrated to have adverse impacts on the ecology of protected dune habitats (Jones et al., 2013; Plassmann et al., 2008; Field et al., 2014). However, the specific impacts of relatively low levels of nutrients from groundwater on aquatic habitats in dune systems have only recently been documented (Rhymes et al., 2014). As well as nutrients, diffuse inputs of dissolved organic matter (DOM) and micro-organisms into groundwater and surface waters also occur via runoff, field drainage and leaching, as a result of agricultural practices such as slurry and fertiliser application. Previously, these diffuse inputs have largely been characterised by using nutrients as a proxy (e.g. Vadas et al., 2007), although more recent studies are now examining diffuse sources and pathways by investigating pathogenic micro-organisms (Kay et al., 2008) and

* Corresponding author.
E-mail address: jenrhymes@ceh.ac.uk (J. Rhymes).

http://dx.doi.org/10.1016/j.scitotenv.2014.12.085
0048-9697/

characterising DOM by natural fluorescence (Hudson et al., 2007). To date there have been no studies combining chemical, fluorescent and microbial techniques to help decipher multiple diffuse sources and pathways. Excitation emission matrix fluorescence spectroscopy (EEMS) can be used to trace DOM from agricultural sources (Baker, 2002; Old et al., 2012). EEMS is sensitive enough to characterise fulvic-like, humic-like and protein-like substances (Tryptophan-like and tyrosine-like) within the DOM to help characterise and quantify the extent of contamination by effluents from different sources (Hudson et al., 2007). Fulvic-like and humic-like substances are derived from the breakdown of plant material (Stedmon et al., 2003), whereas large inputs of tryptophan-like substances are associated with readily biodegradable material from sewage and farm waste slurry (e.g. Baker, 2001). Agricultural diffuse sources such as animal waste are characterised by high protein-like fluorescence with very high ratios of tryptophan-like to fulvic/humic-like fluorescence compared to stream waters (Baker, 2002); these ratios are sensitive enough to characterise inputs from different livestock animals such as pigs and sheep (Baker, 2002).

Currently, the WHO Guidelines for Drinking Water Quality, adopted as standard in many countries, use total coliforms (TC), or specifically *Escherichia coli* (*E. coli*) a sub-group of faecal coliforms, as faecal indicators for the safety of water supplies. In some countries, such as The Netherlands and Denmark, groundwater is abstracted from sand dune systems to supply drinking water, indicating the importance of understanding the fate and occurrence of TC and *E. coli* within these systems (Smeets et al., 2009). The enumeration of TC and *E. coli* is also used as an indicator of water quality within the revised bathing water directive; *E. coli* counts greater than 10,000 counts per 100 mL and TC counts greater than 2000 counts per 100 mL would fail to meet the required standards in the directive (European Communitiy, 2006). Although the TC group includes the species *E. coli*, which is generally considered to be specific for faecal contamination, it also includes other genera such as *Klebsiella* and *Citrobacter* which are not necessarily of faecal origin and can emanate from alternative organic sources such as decaying plant materials and soils (WHO, 2006).

While some *E. coli* represent enteric pathogens (Savageau, 1983), other strains of this species can grow and maintain populations in the environment if the conditions are suitable (Byappanahalli and Fujioka, 2004). Sources of *E. coli* include septic tanks, sewer lines, wastewater treatment plants, manure spreading on land, livestock and wildlife. These sources also contribute DOM and nutrient inputs. Despite advanced wastewater treatment efforts by water treatment companies, some UK bathing sites do not always produce full compliance with microbial standards (Crowther et al., 2002) due to other diffuse sources within catchments, resulting in a greater proportion of nonconformity due to agriculture. More than 150 different pathogens, associated with both environmental and human health risks can be found in livestock manure which can significantly increase bacterial loading to the subsurface, causing contamination within soils, groundwater and stream water (Gerba and Smith, 2005). The transport time and distance travelled by bacteria reaching the groundwater or streams depend on the rate at which bacteria are released from manure, the presence of preferential pathway networks within soil and the depth to the groundwater (Abuashour et al., 1994; Unc and Goss, 2003). The presence of TCs in surface or groundwater is usually considered evidence of recent faecal contamination, with *E. coli* remaining active for 16–45 days in the subsurface (Taylor et al., 2004).

This study aims to use a combination of inorganic chemistry, DOM fluorescence and culturable *E. coli* and TC counts to evaluate the potential sources and pathways of nutrients and contamination to a sand dune site designated for its international nature conservation importance, known to be affected by nutrients from the surrounding agricultural land (Rhymes et al., 2014). Building on the previous study, a further year of bi-monthly sampling was carried out to separately assess the degree of potential contamination and likely sources of contaminants from a) off-site sources entering the site from streams, b) off-site sources entering the site via runoff/overland flow, c) groundwater flowing under the site, and d) on-site sources.

2. Materials and methods

2.1. Field monitoring strategy

Aberffraw sand dune system is part of an internationally designated conservation site in the European Union Natura network, located on the southwest corner of the island of Anglesey in North Wales, UK (53°11′N, 4°27′W). It is designated for its dune habitats, in particular its dune slack wetlands and the rare plant and invertebrate species they support (Curreli et al., 2013). The site is in a low valley surrounded on three sides by agricultural land. The agricultural land is reseeded and fertilised pasture used for sheep and cattle grazing, with feed stations on land immediately adjacent to the south-east dune site edge (Fig. 1). Streams A and B (Fig. 1) drain this heavily fertilised agricultural and both lead onto the site. Flow in stream A is episodic and flows primarily in winter, compared with the permanent and faster flowing stream B. Annual long term average rainfall at the site is 847 mm (Stratford et al., 2013). There are a number of potential pathways by which nutrients and coliforms can enter the site; these include streams and ditches, surface runoff draining agricultural land and flowing onto the site, seepage of nutrients into the groundwater flowing under the site, and on-site sources such as grazing cattle and rabbits. Previous work has previously shown a nitrogen groundwater contamination gradient that extends into the site from the fertilised pastureland on the south east border with groundwater travelling in a south westerly direction (Fig. 1) (Rhymes et al., 2014). To determine the nature and pathways of the contamination, measurements were made bimonthly for a 12 month period (i.e. 6 sample periods) across streams, ditches, standing surface water and groundwater in dune slacks. Stream samples were collected from two streams (A and B) entering the site (Fig. 1). Samples were collected from upstream sampling points (A1 & B1) and downstream sampling points (A2 & B2) by dipping a clean collecting container into the surface flow. Groundwater samples within dune slacks were measured from fifteen groundwater monitoring piezometers across the site, installed to 2 m depth. Four piezometers (Fig. 1, triangles) aimed at looking at impacts from surface runoff. The remaining eleven aimed at evaluating potential gradients in the groundwater of water chemistry, natural fluorescence and TC and *E. coli* abundance with distance from the contamination sources on the south-east edge of the site (Fig. 1, squares). Samples were collected from the top 10 cm of the water table at each well using a sterilised pump and tubing, which was disinfected with Trigene and flushed three times with deionised water between samples into 250 mL sterile plastic bottles. During periods of inundation, for up to 4 months between November and February (Rhymes et al., 2014), when water tables were above ground level in certain slacks, samples of the standing water above the piezometer were taken. Groundwater depth was measured monthly at each piezometer. For fluorescence and *E. coli* measurements, sampling was conducted for 4 of the 6 sampling periods.

2.2. Water chemistry analysis

Samples from piezometers and streams were analysed within 24 h of collection and stored in darkness at 5 °C prior to chemical analysis. In the laboratory groundwater pH was recorded for each sample which was then filtered through a 0.45 μm nylon syringe filter (Avonchem™). Dissolved inorganic anions (fluoride, chloride, nitrite, nitrate, phosphate and sulphate) and cations (sodium, ammonium, potassium, calcium and magnesium) were then quantified on an ion chromatograph (Metrohm, UK Ltd.). Dissolved inorganic nitrogen (DIN) was calculated as the sum of NO_3-N, NO_2-N and NH_4-N. Total nitrogen (TN) and total carbon were analysed by thermal oxidation on a thermalox TOC/TN

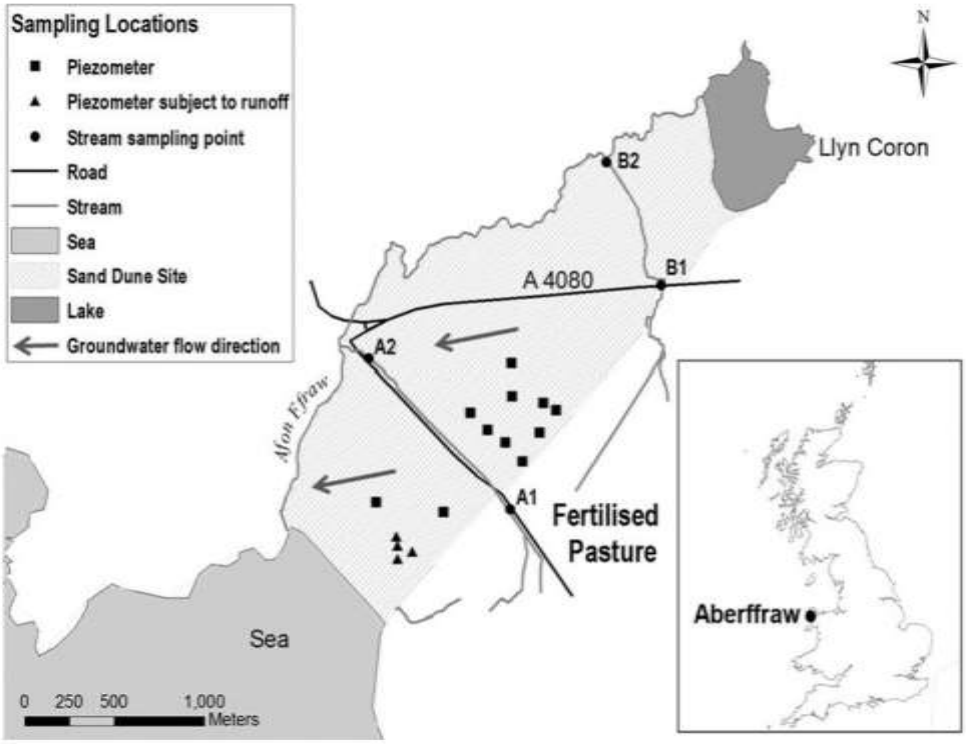


Fig. 1. Map of Aberffraw dune system showing all piezometer and stream sampling points. Cross-hatched area represents designated site. The surrounding white area is agricultural land, predominantly pastureland. Redrawn from Ordnance Survey.

analyser. Total inorganic carbon (TIC) was measured within a TIC-reactor on a thermalox. Dissolved organic nitrogen (DON) was calculated by the difference between TN and calculated DIN and Dissolved Organic Carbon (DOC) was calculated by the difference between TC and TIC.

2.3. Fluorescence analysis

All samples were filtered in the field using 0.45 μm silver membrane filters (Steplitech) and stored in the dark at 5 °C prior to analysis. Analysis took place 48 h after collection at room temperature. Fluorescence measurements were obtained from a spectrophotometer (Variant Cary Eclipse) fitted with a xenon flash lamp using slit widths of 5 nm, an integration time of 12.5 ms and voltage of 700 V. Excitation wavelengths were varied from 200 to 400 nm in steps of 5 nm and emission wavelengths from 280 to 500 nm in steps of 2 nm. Post-processing was carried out using an R script described by Lapworth and Kinniburgh (2009) within the statistical package R. Absorbance was measured in a 1 cm cuvette on a UV–vis spectrophotometer (Varian Cary 50) at 1 nm intervals from 800 to 200 nm and $SUVA^{254}$ was calculated by dividing absorbance at 254 nm by DOC concentration (Weishaar et al., 2003). Absorbance measurements were scatter corrected employing the method of Blough et al. (1993). All florescence data were corrected for instrument effects to account for lamp output, and corrected for inner filter effects using the corrected absorbance data (Lakowicz and Geddes, 1991). The data were reported in standard Raman units, which normalise the intensity by the area under the Raman peak between emission wavelengths of 380–410 for the excitation wavelength of 348 nm.

2.4. Enumeration of TC and E. coli

Water samples were processed within 6 h of collection. *E. coli* and TC counts in water samples were determined in duplicate by filtering 20 mL of water through 0.2 μm cellulose nitrate filters (Whatman). Subsequently, cellulose nitrate filters were aseptically transferred onto Harlequin™ *E. coli*/Coliform Medium (Lab M). Culture plates were incubated for 22 h at 37 °C prior to colony counting: TCs generated purple colonies and *E. coli* produced blue colonies Harlequin™ *E. coli*/Coliform Medium.

2.5. Statistical analysis

All statistical analyses were performed using minitab v.16. Differences in stream water chemistry concentrations, fluorescence concentrations and *E. coli* and TC counts between upstream (A1, B1) and downstream (A2, B2) sampling points in two streams, A and B, were assessed using ANCOVA (Stevens, 1982), where the date of sample collection was used as a covariate to account for seasonal variation.

In order to test for statistical differences in chemical, fluorescence and *E. coli* and TC count variables between streams, runoff and underlying groundwater gradients, annual means of all variables were analysed by grouping piezometers into three classes based on their distance from the south-east site edge (0–150 m, 150–300 m, 300–450 m, excluding piezometers which were subject to run-off), grouping piezometers subject to runoff for March samples alone (see explanation below) and annual mean upstream sampling points from streams A and B (A1 & B1). Statistical tests used analysis of variance. Data that proved not normally distributed (Kolmogorov–Smirnov test) were transformed using a Johnson's transformation, which transforms the data to follow a normal distribution using the Johnson distribution system.

In order to assess runoff inputs separately from any contributions from on-site sources, samples from piezometers subject to runoff (Fig. 1, triangles) were compared with all other piezometers (Fig. 1, squares) for samples collected in March, as this was the only month where all piezometers were subject to groundwater flooding. An ANCOVA (Stevens, 1982) was carried out on these two groups for all water chemistry, *E. coli* and TC counts and fluorescent spectroscopy variables, with distance from the south-east site edge as a covariate to account for potential underlying gradients in water chemistry due to other sources. Data that proved not normally distributed (Kolmogorov–Smirnov test) were transformed using a Johnson's transformation.

In order to assess underlying input gradients via the groundwater into the site, while accounting for runoff inputs, we separately analysed annual means of variables for all piezometers that were not subject to runoff (Fig. 1, squares). Relationships between annual means of all measurements with distance from the south-east site edge were investigated using linear regression. Data that proved not normally distributed (Kolmogorov–Smirnov test) were transformed using a Johnson's transformation.

3. Results

When comparing the water chemistry, *E. coli* and TC counts and fluorescence among the main sources (Table 1), nitrate and DIN concentrations in runoff and streams were significantly higher than those found in groundwater samples from the 150–300 m and 300–450 m distance classes. There was no significant difference in nitrate concentrations between the three distance classes. However, significantly higher concentrations of DIN were observed in the 0–150 m class closest to the south-east site edge compared with the 300–450 m class. *E. coli* counts in the upstream sampling points of streams were significantly higher by an order of magnitude than in the runoff samples and in the groundwater at all distance classes (Table 1). TCs in streams were significantly higher than in the slacks subject to runoff, but were not significantly different from groundwater. There were no significant differences between sampling locations for DOC, phosphate, fulvic like, tryptophan like, TRP:FA and $SUVA^{254}$. All fluorescent TRP:FA ratios measured within surface waters, groundwater and streams throughout the year did not exceed 1 R.U. and are described as uncontaminated drainage waters (Naden et al., 2010).

3.1. Stream nutrient and bacterial attenuation

Upstream annual mean nitrate and DIN concentrations are significantly higher in stream A than in stream B, however *E. coli* counts are alike for both A and B streams for both upstream and downstream sampling points. Annual mean nitrogen concentrations upstream of streams entering the site are high at all stream sampling points (e.g. annual mean 12 mg/L of nitrate and 2.6 mg/L of DIN at B1) but are very high in A1, which drains from the south-east site edge into the site, where concentrations reached a maximum of 39 mg/L of nitrate in January. In stream A, the annual mean concentrations and counts for nitrate, DIN and *E. coli* were significantly higher upstream (A1) than those downstream (A2) (Fig. 2). By contrast, concentrations of nitrate and DIN in stream B (B1) did not decrease downstream to B2. No significance was found for fluorescence variables for all stream sampling points; TRP:FA mean concentrations were A1 = 0.217 ± 0.034 R.U., A2 = 0.280 ± 0.042 R.U., B1 = 0.457 ± 0.203 R.U. and B2 = 0.268 ± 0.042 R.U. Total nitrogen concentrations showed the same significant pattern as nitrate concentrations for all stream sampling points.

3.2. Runoff input

In order to assess the contribution of off-site sources of contamination (cattle feed, overnight dunging, manure or slurry spreading) separately from any contribution by on-site sources (rabbits, dunging of cattle while grazing on-site), slacks only subject to groundwater flooding (Fig. 1, squares) were compared with those experiencing groundwater flooding in addition to runoff from neighbouring fields (Fig. 1, triangles), during a period when both sets of piezometers experienced surface inundation (Table 2). Significantly higher concentrations of nitrate and DIN were observed in those slacks exposed to runoff. However, there was no significant difference for DON, DOC, *E. coli* and TC counts, fluorescent variables and all other variables measured (Table 2).

3.3. Underlying gradients of nitrogen input via groundwater and on-site inputs

In order to assess underlying input gradients via the groundwater into the site, while accounting for runoff inputs, we separately analysed annual means of variables for all piezometers that were not subject to runoff against distance from potential source area at the south-east site edge (Fig. 3). There were significant gradients of declining nitrate, DIN and DON concentrations into the site, away from their likely source at the south-east site edge (significant negative regression Fig. 3; a) Nitrate Coef = −0.005, b) DON Coef = −0.001 and c) DIN Coef = −0.), with nitrate and DIN decreasing very strongly. A trend of linear decline was apparent for DOC concentrations but this was not significant (Fig. 3d). Fig. 4 shows the spatial pattern of *E. coli* and TC counts and tryptophan-like and fulvic-like fluorescence. Counts of *E. coli* (Figs. 3e and 4b) and TC (Fig. 4a) observed across the site showed no correlation with distance from the south-east site edge (Log *E. coli* counts/100 mL; max = 4.6, min = 0.00 and Log TC counts/100 mL; max = 4.85, min = 0.00). Tryptophan like fluorescence and fulvic like fluorescence (Fig. 3f and g) also showed no correlation with distance from the south-east south edge. However the TRP:FA ratio (Fig. 3h) showed a strong positive significant trend for increasing TRP:FA ratio away from the south-east site edge.

4. Discussion

In this study we show that a combined analysis of nutrient concentrations, fluorescence and microbial abundance can help identify potential sources and pathways of nutrients and *E. coli* and TC inputs impacting a wetland site of international nature conservation

Table 1
Summary of annual mean water chemistry, *E. coli* and TC counts and fluorescence concentrations and counts for upstream sampling points for two streams (A and B), mean standing water for flooded slacks subject to runoff in March and for annual mean groundwater and standing water for distance classes (categorised piezometers located 0–150 m, 150–300 m and 300–450 m away from the south east site edge, excluding piezometers subject to runoff). Values for each variable are expressed as mean ± standard error. Significant differences among classes are shown in bold; values with the same letter are not significantly different to each other.

Variable		Stream	Groundwater and standing water			
			Runoff	Distance from site edge (m)		
				0–150	150–300	300–450
Water chemistry (mg/L)	Nitrate	**20.821 ± 9.763^{A}**	**7.519 ± 1.556^{A}**	**3.197 ± 1.516^{AB}**	**0.033 ± 0.019^{B}**	**0.018 ± 0.007^{B}**
	DIN	**4.720 ± 2.209^{A}**	**1.702 ± 0.352^{A}**	**0.756 ± 0.346^{AC}**	**0.050 ± 0.019^{BC}**	**0.017 ± 0.003^{B}**
	DON	**0.987 ± 0.459^{A}**	**0.658 ± 0.042^{AB}**	**0.430 ± 0.076^{AB}**	**0.322 ± 0.088^{B}**	**0.249 ± 0.034^{B}**
	DOC	8.492 ± 13.903	24.600 ± 13.903	14.250 ± 0.966	7.789 ± 4.878	8.602 ± 1.863
	Phosphate	0.750 ± 0.654	0.009 ± 0.003	0.020 ± 0.003	0.012 ± 0.001	0.015 ± 0.005
Bacterial counts (Log 10 counts per 100 mL)	*E. coli*	**3.458 ± 0.033^{A}**	**0.000 ± 0.000^{B}**	**0.151 ± 0.151^{B}**	**0.403 ± 0.242^{B}**	**0.285 ± 0.133^{B}**
	TC	**4.087 ± 0.197^{A}**	**2.559 ± 0.169^{B}**	**2.863 ± 0.083^{AB}**	**2.793 ± 0.355^{AB}**	**2.641 ± 0.431^{AB}**
Fluorescence (R.U.)	Fulvic like	1.423 ± 0.092	1.491 ± 0.003	1.321 ± 0.048	1.367 ± 0.078	1.251 ± 0.041
	Tryptophan like	1.141 ± 0.030	1.167 ± 0.002	1.114 ± 0.015	1.123 ± 0.024	1.097 ± 0.016
	TRP:FA	0.810 ± 0.036	0.782 ± 0.001	0.844 ± 0.019	0.838 ± 0.027	0.888 ± 0.017
Absorbance (L mg^{-1} m^{-1})	$SUVA^{254}$	0.043 ± 0.011	0.061 ± 0.046	0.018 ± 0.003	0.020 ± 0.003	0.015 ± 0.005

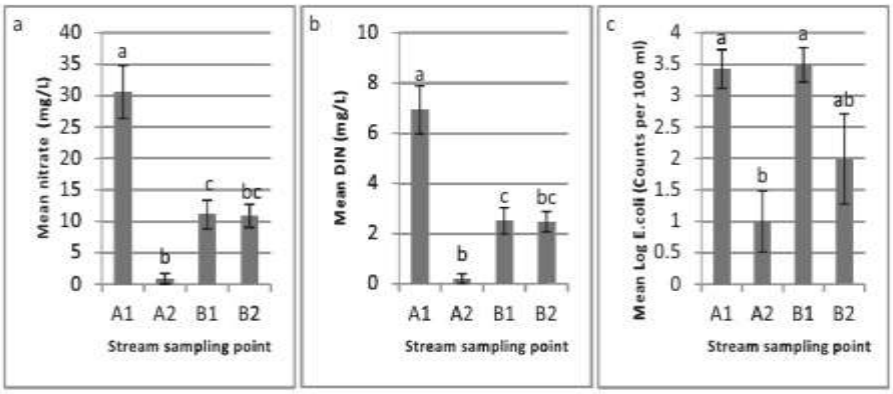


Fig. 2. Annual mean concentrations for nutrients and *E. coli* counts of two streams (A and B) entering the site at upstream and downstream sampling points, showing: a) Nitrate b) DIN and c) Log *E. coli* counts. Letters denote significant differences between stream sampling points A1 & B1 upstream and A2 & B2 downstream – see Fig. 1.

importance. The multiple pathways and the fate of nutrients and *E. coli* and TC to the site are summarised in Fig. S1 in the Supplementary Material.

4.1. Off-site sources (fertiliser, cattle dung, slurry and fertilisers applied to fields)

4.1.1. Streams

Streams A and B have similar annual mean *E. coli* abundances as they enter the site, suggesting that both streams have similar faecal inputs from grazers on adjacent pastureland. By contrast, nitrate concentrations in Stream A, which drains the pastureland and flows onto the sand dune site, are significantly higher than those in stream B and previous studies have shown that they can exceed the 50 mg/L threshold for designation of a nitrate vulnerable zone by the UK Environment Agency (Rhymes et al., 2014; Environment Agency, 2012). This implies that stream A has additional N inputs compared to stream B; these are likely to be from fertilisers leaching from the steep sloped pastureland adjoining the stream. This is a result of the lack of significant difference in TRP:FA ratio between the two streams suggesting a common contamination source from animal dung, with differences in nutrient loading due to fertiliser inputs only.

The attenuation of nitrate concentrations in stream A is probably caused by a combination of processes, including in-stream microbial denitrification, plant uptake, seepage through the river bank, dilution during high groundwater levels and transient storage (Mulholland et al., 2008). The lack of nutrient attenuation in stream B may be due to the absence of nitrophilous species such as *Phragmites australis* (Stamati et al., 2010) and the faster stream flow in stream B which reduces the water-sediment contact (Peterson et al., 2001) and reduces the ability for microbes to assimilate nitrogen from the water column (Grimm and Fisher, 1989). Similarly *E .coli* counts decrease from upstream to downstream sampling points in stream A but not stream B. Studies have shown that within sediment there are higher populations of TCs than the overlaying water (Smith et al., 2008) as sediments serve as a hospitable environment for bacterial survival due to the availability of organic matter (Jamieson et al., 2005) suggesting that *E. coli* may be being deposited into the stream and incorporated within the sediment. Subsequently during storm events bacteria can be re-suspended into the water column and continue to flow downstream (Jeng et al., 2005), posing a potential threat to bathing waters on the sandy beach at Aberffraw at the mouth of the river Ffraw since mean *E. coli* colonies within both streams exceed 2000 colonies per 100 mL which would fail to meet the required standards for the EU bathing water directive (2006).

Table 2
Summary of selected chemical, fluorescent and *E. coli* and TC count variables for piezometers subject to both runoff and groundwater flooding and wells subject to groundwater flooding alone in March. Values for each variable are expressed as mean ± standard error. Significant differences between groups of slacks are shown in bold and denoted by letters.

Variable		Slacks subject to:	
		Runoff and groundwater flooding	Groundwater flooding
Water chemistry (mg/L)	Nitrate	**7.519 ± 1.556^{A}**	**0.676 ± 0.386^{B}**
	DIN	**1.702 ± 0.352^{A}**	**0.181 ± 0.0920^{B}**
	DON	0.658 ± 0.042	0.505 ± 0.072
	DOC	11.100 ± 4.700	19.117 ± 2.023
	Phosphate	0.014 ± 0.009	0.024 ± 0.008
Bacterial counts (Log 10 counts per 100 mL)	*E. coli*	0.000 ± 0.000	0.841 ± 0.351
	TC	3.036 ± 0.301	3.370 ± 0.086
Fluorescence spectroscopy (R.U.)	Fulvic like	1.491 ± 0.003	1.515 ± 0.074
	Tryptophan like	1.167 ± 0.002	1.172 ± 0.023
	TRP:FA	0.782 ± 0.001	0.785 ± 0.024
Absorbance ($L\ mg^{-1}\ m^{-1}$)	$Suva^{254}$	0.061 ± 0.046	0.018 ± 0.003

4.1.2. Surface runoff

The nature of the nitrate contamination contributing via runoff is also likely to be due to applied fertilisers on the south-east pastureland, rather than nutrients from slurry and dung. Nitrate concentrations are eleven times higher in slacks subject to runoff and groundwater flooding compared to piezometers subject to groundwater flooding alone, whereas the TRP:FA fluorescence ratios were <1 R.U. which are described as uncontaminated drainage waters compared to the described slurry TRP:FA fluorescence ranging from 2–5 R.U. (Naden et al., 2010). In support of this, negligible *E. coli* and TC counts were observed in slacks subject to runoff which would have been expected if the contamination resulted from slurry application (Thurston-Enriquez et al., 2005). Surface runoff events are sporadic as they are caused by heavy periods of rainfall, nevertheless while nutrient concentrations are high during runoff events and in the subsequent standing surface water, the concentrations in groundwater return to low concentrations once the runoff ceases. This may be a result of denitrification caused by the increased availability of nitrate and anaerobic conditions (Mulvaney et al., 1997), or due to plant and microbial uptake highlighting the function of dune systems in filtering nutrients. However, the initial nitrate and DIN concentrations in the standing water are much higher than the levels of 0.2 mg DIN L^{-1} above which biological effects have been determined previously at the site (Rhymes et al., 2014), suggesting adverse impacts on the site due to nutrients from this source. In addition, the total flux of nitrogen entering the site as a result of these runoff events is also unknown. Calculating this input may help harmonise dose-related critical load approaches to damage (Bobbink and Hettelingh, 2010) with concentration based methods used in aquatic systems (Camargo and Alonso, 2006).

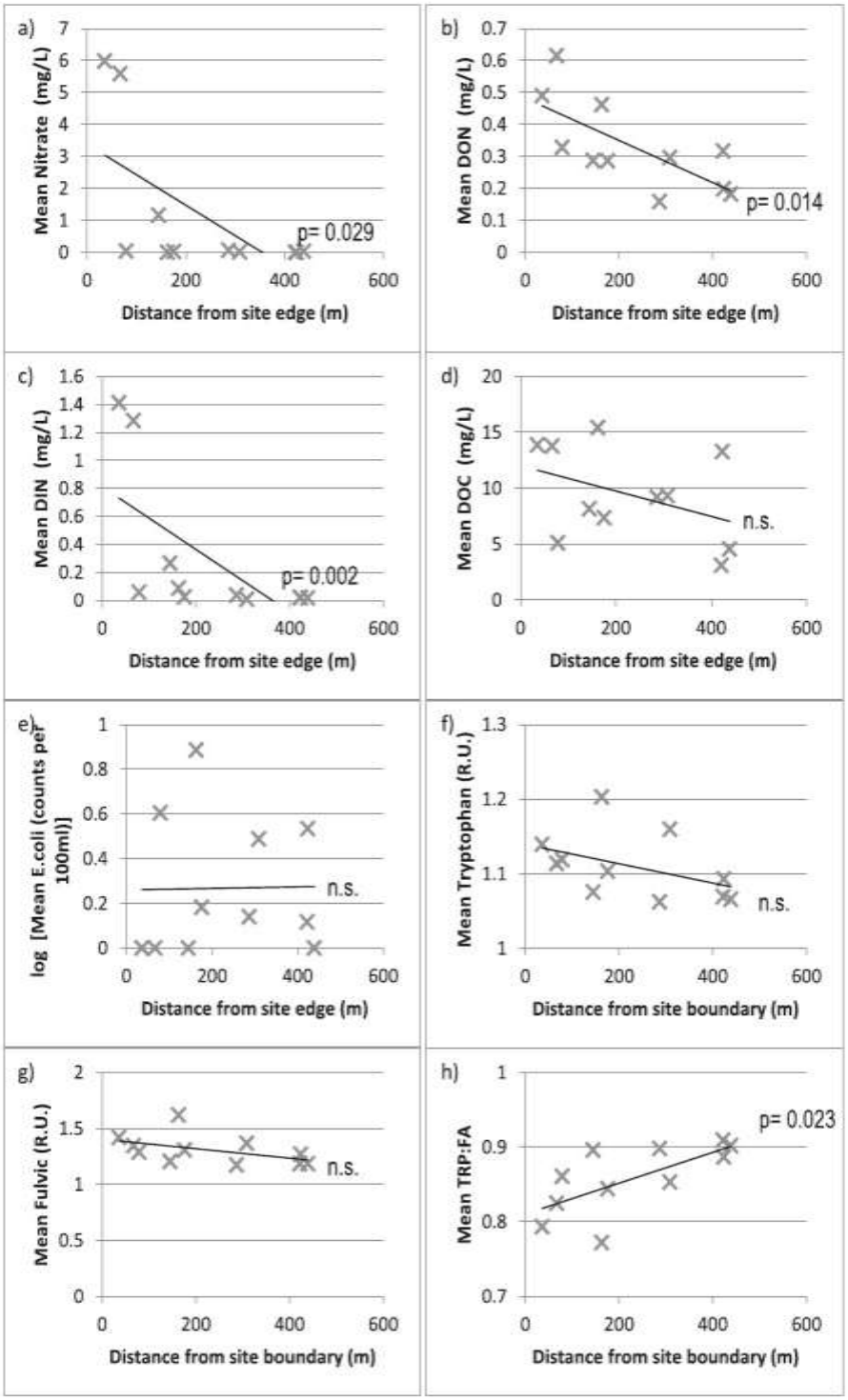


Fig. 3. Relationships between annual mean groundwater a) nitrate, b) DON, c) DIN, d) DOC, e) *E. coli*, f) tryptophan-like, g) fulvic-like, and h) TRP:FA with distance from south-east site edge. Trendline for all variables is linear.

4.1.3. Groundwater

Nutrients, likely to be from ammonium nitrate fertiliser, are entering the site by leaching through sandy agricultural soils with a low water holding capacity (Skiba and Wainwright, 1984), and subsequently flowing under the site via the groundwater. As a result a nitrate, DIN and DON groundwater gradient was found independent of runoff influence, confirming that the gradients of elevated nutrients in groundwater and soils observed in an earlier study were not due to surface flooding (Rhymes et al., 2014). There was no gradient in *E. coli* and TC counts within the groundwater under the site, suggesting that the higher concentrations of *E. coli* and TCs originating in the south-east pasture and observed in the streams are probably filtered out by the sandy soils during recharge transit in the subsurface before reaching the site (Price et al., 2013).

4.2. On-site sources (rabbits, cattle dung)

E. coli and TCs were found across the site at low levels, but showed no relationship with the distance from the south-east site edge. Similarly, the tryptophan like fluorescence showed no relationship with the distance from the south-east site edge. This suggests that the main source of groundwater *E. coli* and TCs derive from on-site cattle and rabbits. Therefore, in addition to streambed seepage, runoff and underlying groundwater nutrient inputs, there are nutrient and *E. coli* and TC inputs

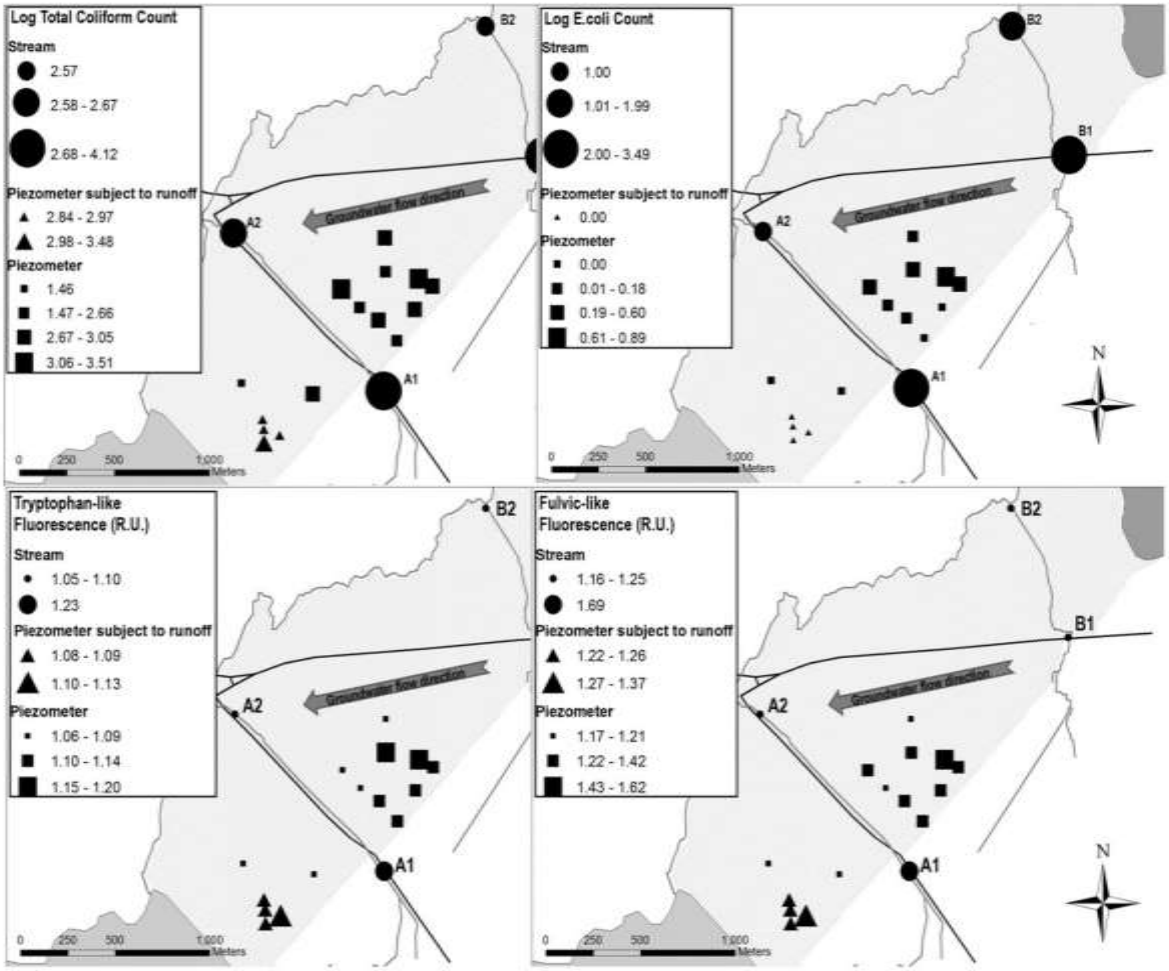


Fig. 4. Spatial variation of a) Log *E. coli* counts/100 mL, b) Log total coliform counts/100 mL, c) tryptophan-like fluorescence (R.U.) and d) fulvic-like fluorescence (R.U.) for streams (annual mean), piezometers subject to runoff (March counts and concentrations) and piezometers (annual mean).

from on-site grazers such as cattle and rabbits. Despite these small-scale on-site inputs, all piezometers across the site meet the mandatory bathing water directive standards of 2000 *E. coli* counts per 100 mL.

5. Conclusions

The combination of chemical, fluorescent and microbial techniques has helped identify potential nutrient sources from fertilisers and grazers (Baker, 2002). The findings of this study suggest that nutrients are being attenuated and processed within the site thereby providing a valuable ecosystem service. However at the same time, the influx of nutrients is likely to have an adverse effect on the dune slack ecology, with impacts on plant community composition (Rhymes et al., 2014). The analysis of surface waters, slacks and piezometers across the site has allowed the differentiation between the input pathways of streams, runoff events, underlying groundwater nutrient gradients and on-site grazing inputs. While the study was able to distinguish multiple pathways, the full potential of the techniques to differentiate between livestock sources (e.g. sheep, cattle, pigs) was not explored in this study. This combination of techniques provides an approach which could allow for a detailed understanding of nutrient contamination sources and pathways relatively cheaply. Such information is key for designing successful management plans to reduce the inputs of contaminants which might be having detrimental effects on sites of conservational value. It could also be implemented for other applications such as tracking faecal sources within bathing waters and fisheries zones, as currently the standard enumeration of FIB does not distinguish between human or other animal sources of contamination, and methods that do so are expensive (Field and Samadpour, 2007).

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.12.085.

References

Abuashour, J., Joy, D.M., Lee, H., Whiteley, H.R., Zelin, S., 1994. Transport of microorganisms through soil. Water Air Soil Pollut. 75, 141–158.
Baker, A., 2001. Fluorescence excitation-emission matrix characterization of some sewage-impacted rivers. Environ. Sci. Technol. 35, 948–953.
Baker, A., 2002. Fluorescence properties of some farm wastes: implications for water quality monitoring. Water Res. 36, 189–195.
Blough, N.V., Zafiriou, O.C., Bonilla, J., 1993. Optical-absorption spectra of waters from the Orinoco River outflow — terrestrial input of colored organic-matter to the Caribbean. J. Geophys. Res. Oceans 98, 2271–2278.
Bobbink, R., Hettelingh, J.-P., 2010. Review and revision of empirical critical loads and dose–response relationships. Proceedings of an Expert Workshop, Noordwijkerhout, pp. 23–25.
Byappanahalli, M., Fujioka, R., 2004. Indigenous soil bacteria and low moisture may limit but allow faecal bacteria to multiply and become a minor population in tropical soils. Water Sci. Technol. 50, 27–32.
Camargo, J.A., Alonso, A., 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environ. Int. 32, 831–849.
Crowther, J., Kay, D., Wyer, M.D., 2002. Faecal-indicator concentrations in waters draining lowland pastoral catchments in the UK: relationships with land use and farming practices. Water Res. 36, 1725–1734.
Curreli, A., Wallace, H., Freeman, C., Hollingham, M., Stratford, C., Johnson, H., Jones, L., 2013. Eco-hydrological requirements of dune slack vegetation and the implications of climate change. Sci. Total Environ. 443, 910–919.
Environment Agency, 2012. Method statement for nitrate vulnerable zone review. Surface Waters Environment Agency Report to Defra and Welsh Government — Supporting Paper for the Implementation of the Nitrates Directive 2013–2016.

European Community, 2006. Directive 2006/7/EC of the European Parliament and of the Council of 15 February (2006) concerning the management of bathing water quality and repealing Directive 76/160/EEC. Official Journal of the European Union, L64/37–51.
Field, K.G., Samadpour, M., 2007. Fecal source tracking, the indicator paradigm, and managing water quality. Water Res. 41, 3517–3538.
Field, C.D., Dise, N.B., R.J., P., Britton, A.J., Emmett, B.A., Helliwell, R.C., Hughes, S., Jones, L., Lees, S., Leake, J.R., Leith, I.D., Phoenix, G.K., Power, S.A., Sheppard, L.J., Southon, G.E., Stevens, C.J., Caporn, S.J.M., 2014. The role of nitrogen deposition in widespread plant community change across semi-natural habitats. Ecosystems 17, 864–877.
Fowler, D., O'Donoghue, M., Muller, J., Smith, R., Dragosits, U., Skiba, U., Sutton, M., Brimblecombe, P., 2005. A chronology of nitrogen deposition in the UK between 1900 and 2000. Water Air Soil Pollut. Focus 4, 9–23.
Galloway, J.N., Cowling, E.B., 2002. Reactive nitrogen and the world: 200 years of change. Ambio 31, 64–71.
Gerba, C.P., Smith, J.E., 2005. Sources of pathogenic microorganisms and their fate during land application of wastes. J. Environ. Qual. 34, 42–48.
Grimm, N.B., Fisher, S.G., 1989. Stability of periphyton and macroinvertebrates to disturbance by flash floods in a desert stream. J. N. Am. Benthol. Soc. 8, 293–307.
Hooda, P.S., Edwards, A.C., Anderson, H.A., Miller, A., 2000. A review of water quality concerns in livestock farming areas. Sci. Total Environ. 250, 143–167.
Hudson, N., Baker, A., Reynolds, D., 2007. Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters — a review. River Res. Appl. 23, 631–649.
Jamieson, R., Joy, D.M., Lee, H., Kostaschuk, R., Gordon, R., 2005. Transport and deposition of sediment-associated *Escherichia coli* in natural streams. Water Res. 39, 2665–2675.
Jeng, H.W.C., England, A.J., Bradford, H.B., 2005. Indicator organisms associated with stormwater suspended particles and estuarine sediment. J. Environ. Sci. Health. Part A Toxic/Hazard. Subst. Environ. Eng. 40, 779–791.
Jones, L., Nizam, M., Reynolds, B., Bareham, S., Oxley, E., 2013. Upwind impacts of ammonia from an intensive poultry unit. Environ. Pollut. 180, 221–228.
Kay, D., Crowther, J., Fewtrell, L., Francis, C.A., Hopkins, M., Kay, C., McDonald, A.T., Stapleton, C.M., Watkins, J., Wilkinson, J., Wyer, M.D., 2008. Quantification and control of microbial pollution from agriculture: a new policy challenge? Environ. Sci. Pol. 11, 171–184.
Lakowicz, J.R., Geddes, C.D., 1991. Topics in Fluorescence Spectroscopy. Springer.
Lapworth, D.J., Kinniburgh, D.G., 2009. An R script for visualising and analysing fluorescence excitation–emission matrices (EEMs). Comput. Geosci. 35, 2160–2163.
Mulholland, P.J., Helton, A.M., Poole, G.C., Hall, R.O., Hamilton, S.K., Peterson, B.J., Tank, J.L., Ashkenas, L.R., Cooper, L.W., Dahm, C.N., Dodds, W.K., Findlay, S.E.G., Gregory, S.V., Grimm, N.B., Johnson, S.L., McDowell, W.H., Meyer, J.L., Valett, H.M., Webster, J.R., Arango, C.P., Beaulieu, J.J., Bernot, M.J., Burgin, A.J., Crenshaw, C.L., Johnson, L.T., Niederlehner, B.R., O'Brien, J.M., Potter, J.D., Sheibley, R.W., Sobota, D.J., Thomas, S.M., 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452, 202-U46.
Mulvaney, R.L., Khan, S.A., Mulvaney, C.S., 1997. Nitrogen fertilizers promote denitrification. Biol. Fertil. Soils 24, 211–220.
Naden, P.S., Old, G.H., Eliot-Laize, C., Granger, S.J., Hawkins, J.M.B., Bol, R., Haygarth, P., 2010. Assessment of natural fluorescence as a tracer of diffuse agricultural pollution from slurry spreading on intensely-farmed grasslands. Water Res. 44, 1701–1712.
Novotny, V., 1999. Diffuse pollution from agriculture — a worldwide outlook. Water Sci. Technol. 39, 1–13.
Oakes, D.B., Young, C.P., Foster, S.S.D., 1981. The effects of farming practices on groundwater quality in the United-Kingdom. Sci. Total Environ. 21, 17–30.
Old, G.H., Naden, P.S., Granger, S.J., Bilotta, G.S., Brazier, R.E., Macleod, C.J.A., Krueger, T., Bol, R., Hawkins, J.M.B., Haygarth, P., Freer, J., 2012. A novel application of natural fluorescence to understand the sources and transport pathways of pollutants from livestock farming in small headwater catchments. Sci. Total Environ. 417, 169–182.
Peterson, B.J., Wollheim, W.M., Mulholland, P.J., Webster, J.R., Meyer, J.L., Tank, J.L., Marti, E., Bowden, W.B., Valett, H.M., Hershey, A.E., McDowell, W.H., Dodds, W.K., Hamilton, S.K., Gregory, S., Morrall, D.D., 2001. Control of nitrogen export from watersheds by headwater streams. Science 292, 86–90.
Plassmann, K., Brown, N., Jones, M.L.M., Edwards-Jones, G., 2008. Can atmospheric input of nitrogen affect seed bank dynamics in habitats of conservation interest? The case of dune slacks. Appl. Veg. Sci. 11, 413–420.
Price, W.D., Burchell, M.R., Hunt, W.F., Chescheir, G.M., 2013. Long-term study of dune infiltration systems to treat coastal stormwater runoff for fecal bacteria. Ecol. Eng. 52, 1–11.
Rhymes, J., Wallace, H., Fenner, N., Jones, L., 2014. Evidence for sensitivity of dune wetlands to groundwater nutrients. Sci. Total Environ. 490C, 106–113.
Savageau, M.A., 1983. *Escherichia coli* habitats, cell-types, and molecular mechanisms of gene-control. Am. Nat. 122, 732–744.
Skiba, U., Wainwright, M., 1984. Nitrogen transformation in coastal sands and dune soils. J. Arid Environ. 7, 1–8.
Smeets, P., Medema, G., Van Dijk, J., 2009. The Dutch secret: how to provide safe drinking water without chlorine in the Netherlands. Drinking Water Eng. Sci. 2, 1–14.
Smith, J., Edwards, J., Hilger, H., Steck, T.R., 2008. Sediment can be a reservoir for coliform bacteria released into streams. J. Gen. Appl. Microbiol. 54, 173–179.
Stamati, F.E., Chalkias, N., Moraetis, D., Nikolaidis, N.P., 2010. Natural attenuation of nutrients in a Mediterranean drainage canal. J. Environ. Monit. 12, 164–171.
Stedmon, C.A., Markager, S., Bro, R., 2003. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. Mar. Chem. 82, 239–254.
Stevens, J.P., 1982. The analysis of covariance and alternatives — Huitema, Be. Contemp. Psychol. 27, 473–473.
Stratford, C.J., Robins, N.S., Clarke, D., Jones, L., Weaver, G., 2013. An ecohydrological review of dune slacks on the west coast of England and Wales. Ecohydrology 6, 162–171.
Taylor, R., Cronin, A., Pedley, S., Barker, J., Atkinson, T., 2004. The implications of groundwater velocity variations on microbial transport and wellhead protection — review of field evidence. FEMS Microbiol. Ecol. 49, 17–26.
Thurston-Enriquez, J.A., Gilley, J.E., Eghball, B., 2005. Microbial quality of runoff following land application of cattle manure and swine slurry. J. Water Health 3, 157–171.
Unc, A., Goss, M.J., 2003. Movement of faecal bacteria through the vadose zone. Water Air Soil Pollut. 149, 327–337.
Vadas, P.A., Harmel, R.D., Kleinman, P.J.A., 2007. Transformations of soil and manure phosphorus after surface application of manure to field plots. Nutr. Cycl. Agroecosyst. 77, 83–99.
Weishaar, J.L., Aiken, G.R., Bergamaschi, B.A., Fram, M.S., Fujii, R., Mopper, K., 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environ. Sci. Technol. 37, 4702–4708.
WHO, 2006. Guidelines for Drinking-water Quality: First Addendum to Volume 1, Recommendations. World Health Organization.
Withers, P.J.A., Jarvie, H.P., Hodgkinson, R.A., Palmer-Felgate, E.J., Bates, A., Neal, M., Howells, R., Withers, C.M., Wickham, H.D., 2009. Characterization of phosphorus sources in rural watersheds. J. Environ. Qual. 38, 1998–2011.

APPENDIX III: **Poster presented at The Joint Aquatic Science Meeting.**

Evidence for sensitivity of dune wetlands to groundwater nutrients

J. Rhymes*[1], H. Wallace [3], N. Fenner [1] L. Jones [2]

[1]Bangor University, UK; [2]Centre for Ecology and Hydrology, UK; [3] Ecological surveys (Bangor), UK

Introduction

Dune slacks are seasonal wetlands, high in biodiversity, subject to many pressures including climate change, land use change and eutrophication. Despite their biological importance and the threats facing them, the hydrological and nutrient controls are poorly understood. This is the first empirical study to date testing for biological effects in dune systems resulting from groundwater nutrients at low concentrations (Rhymes et al., 2014).

Fig 1- Aberffraw dune system, showing all piezometers and stream (S2 - S9) sampling points.

Site Description

- Aberffraw is located on Anglesey in North Wales.
- Surrounded by highly fertile farmland
- Rivers drain the agricultural area and lead onto the site
- Groundwater flows in a south westerly direction

Hypotheses

- Does nutrient contamination extend into the groundwater under the dune system?
- Is there any evidence in the plant assemblages and soils of dune slacks that these nutrients are accessible to the vegetation in the dune slacks?
- Do they have an adverse ecological impact on the plant community composition?

Gradient analysis

We examined the impact of groundwater nutrients on

- **Monthly water chemistry**
- **Soil chemistry**
- **Vegetation composition**

...of dune slacks at three distance classes (0-150m, 150-300 m, 300–450 m) away from (off-site) nutrient sources.

Contact: Jenny Rhymes
Bangor University
[email]
or
Laurence Jones
[email]

Rhymes, J., Fenner, N., Jones, L., Wallace, H. 2014. Evidence for sensitivity of dune wetlands to groundwater nutrients. Science of the Total Environment.

Results and Discussion

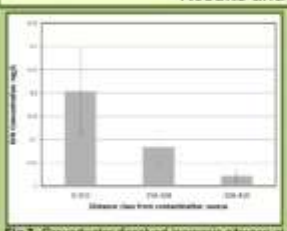


Fig 3- Groundwater dissolved inorganic nitrogen at three distance classes from contamination

Contamination gradient across the site has been found for:

- **Soil nitrite**
- **Groundwater dissolved inorganic nitrogen**
- **Groundwater nitrate**

Main rooting zones of wet and dry slacks are exposed to groundwater for similar periods of the year and therefore are **equally** vulnerable to groundwater contamination.

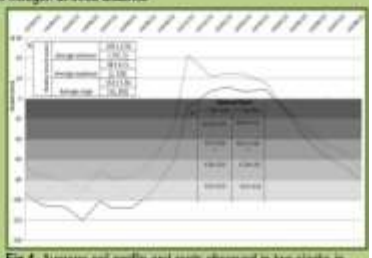
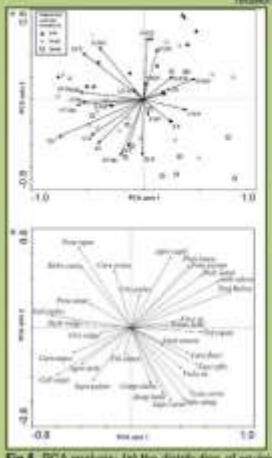


Fig.4- Average soil profile and roots observed in two slacks in relation to groundwater depth over a year.



- Axis 1: **hydrological gradient**
- Axis 2: **soil development/nutrient**
- Axis 2 segregates the 0-150 m class from the 300-450 m class, with quadrats in the 0-150 m class located higher on axis 2.

Vegetation Response

- High axis 2 scores were revealed by **higher fertility species**
- Low axis 2 scores were shown by species with **low fertility** and **higher base** status demand

Fig 5- PCA analysis: **(a)** the distribution of environmental variables with PCA scores, quadrats coded by distance to south-east site boundary. **(b)** The distribution of species with PCA scores.

Hydrological variables	Annual maximum water level (m)	8.80%	**
Water chemistry	$W.NO_3$ (mg/l)	9.00%	**
Combined model	Hydrological parameters and groundwater nitrate (min, max, range, $W.NO_3$	24%	***

Table 1- Environmental variables illustrating percentage of total species variation explained within RDA and significance, when tested singly and within a model

Conclusions

- Off site contamination causes a nutrient gradient in groundwater under the site.
- Nutrients in groundwater affect both soils and vegetation on the site.
- Nitrogen influenced species composition independently of hydrological variables.
- There was clear biological impact at low groundwater nutrient concentrations (mean 0.204 mg/L +/- 0.091 of DIN) (greater abundance of nitrophilous species and fewer basiphoious species).
- Biological impact occurred below previously suggested DIN thresholds of 0.20 – 0.40 (mg/L).

APPENDIX IV: **Supplementary material**

Table A1: Summary of full species names from abbreviated species names in Fig 2.4.

Shortened species name	Full Species name
Agro capil	*Agrostis capillaris*
Agro stoli	*Agrostis stolonifera*
Anag tenel	*Anagallis tenella*
Anth odora	*Anthoxanthum odoratum*
call cusp	*Calliergon cuspidatum*
Camp stella	*Campylium stellatum*
Care arena	*Carex arenaria*
Care flacc	*Carec flacca*
Care nigra	*Carex nigra*
Cirs palus	*Cirsium palustre*
Cirs vulg	*Cirsium vulgare*
Equi offic	*Equisetum offiicinalis*
Equi palust	*Equisetum palustre*
Equi varie	*Equisetum variegatum*
Fest rubra	*Festuca rubra*
Fili ulmar	*Filipendula ulmaria*
Gali palus	*Galium palustre*
Gent amare	*Gentianella amarella*
Holc lanat	*Holcus lanatus*
Homa lutes	*Homalothecium lutescens*
Lotu corni	*Lotus corniculatus*
Plan lance	*Plantago lanceolata*
Pote anser	*Potentilla anserina*
Pote repta	*Potentilla reptans*
Pseu purum	*Pseudoscleropodium purum*
Rubu caesi	*Rubus caesius*
Sela selag	*Selaginella selaginoides*
Trif repen	*Trifolium repens*
Vero sp	*Veronica chamaedrys*
Viola cu	*Viola curtisii*

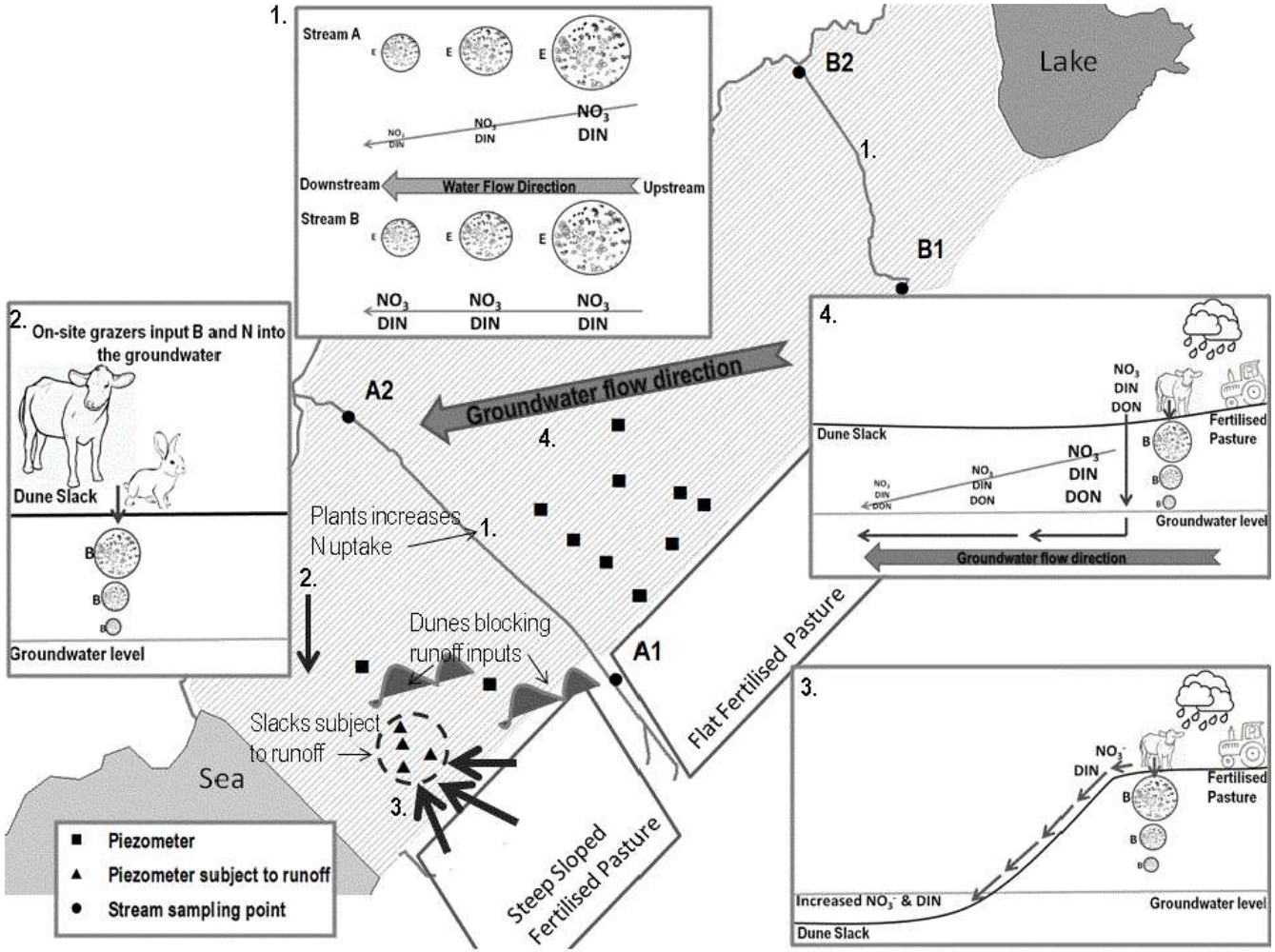


Fig A1 Summary model of nutrient and bacterial loading and pathways within Aberffraw sand dune system. E: *E. coli*; B: Bacteria.

APPENDIX V: **Greenhouse gases-linearity**

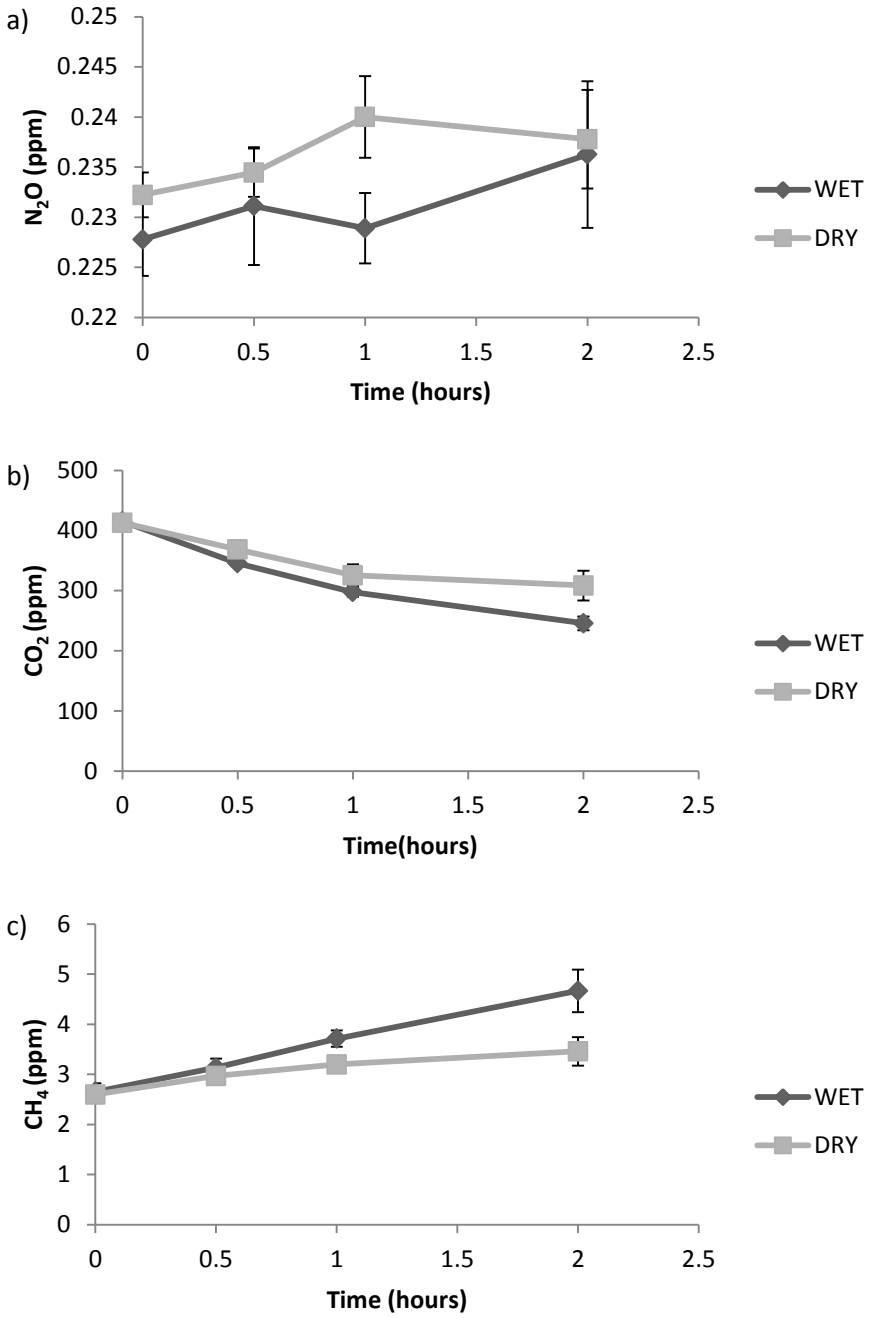


Fig A2 a) N_2O, b) CO_2, and, c) CH_4 concentrations over time to determine sampling incubation periods within mesocosms (Chapter 4).

APPENDIX VI: **Un-presented additional research**

The aim of this data collection was to investigate the ability of sand dune systems to attenuate pathogenic bacteria, and subsequently provide an ecosystem service, namely preventing from bacteria leaching into bathing waters. The sampling method is described in chapter 3.

Methods for DNA extraction and multiplex PCR:

DNA Extraction

Within 24 hours of water samples being collected from all wells and stream sampling points (excluding well 8 and 10) in June, 150 ml were filtered through 0.2 µm polycarbonate membranes (Pall corporation) and stored in lysing matrix E tubes (Obiogene, Montreal, Canada) with 0.5 g of glass beads at -80 °C until DNA extractions were carried out. DNA extraction was then carried out using a modified phenol-chloroform nucleic acid extraction method described by Griffiths et al. (2000), extracted nucleic acid was additionally treated with RNase A (Sigma) at a final concentration of 100 g ml^{-1}.The DNA concentrations were then determined using Nanodrop UV-vis spectrophotometry (Thermo Scientific, USA).

Multiplex PCR

The amplification was performed on 1 µl of purified extraction DNA (20 ng) by adding 24 µl of a reaction mixture which contained 5 µl of MyTaq red reaction buffer (Bioline), 0.25 µl MyTaq HS DNA polymerase (Biloline), 1 µl of each primer (Table A1) and 8.75 µl of PCR water for each sample in a final reaction volume of 25 µl. The amplification was then performed in a PCR system with a denaturation step at 95 °C for 1 min followed by 35 cycles of 95 °C for 15 s, annealing at 60 °C for 15 s and elongation at 72 °C for 1min 30 s. The PCR products were then visualised by gel electrophoresis on 100 ml 2% agarose gel with 6 µl of safeview.

Table A2: PCR primers and products for the detection of virulence genes.

Gene	Virulence Factor	Primer name	Oligonucleotide sequence (5' to 3')	Amplicon size (bp)
asa1	Aggregation substance	ASA 11 ASA 12	GCACGCTATTACGAACTATGA TAAGAAAGAACATCACCACGA	375
gelE	Gelatinase	GEL 11 GEL 12	TATGACAATGCTTTTTGGGAT AGATGCACCCGAAATAATATA	213
cyLA	Cytolisin	CYT 1 CYT IIb	ACTCGGGGATTGATAGGC GCTGCTAAAGCTGCGCTT	688
esp	Enterococcal surface protein	ESP 14F ESP 12R	AGATTTCATCTTTGATTCTTGG AATTGATTCTTTAGCATCTGG	510
hyl	Hyaluronidase	HYL n1 HYL n2	ACAGAAGAGCTGCAGGAAATG GACTGACGTCCAAGTTTCCAA	276

Results:

Results demonstrated here in Fig A2 show that the pathogenic strains tested for are not present within our samples (piezometers and streams), however, our results are smeared, which is likely to be as a result of very high concentrations of DNA, therefore these results must be disregarded. In order to achieve future eligible results, these methods require further optimisation before being applied (e.g. DNA extraction). Nonetheless, the use of these techniques within this application could reveal site specific ecosystem services, where dune systems attenuate pathogenic bacteria from anthropogenic sources.

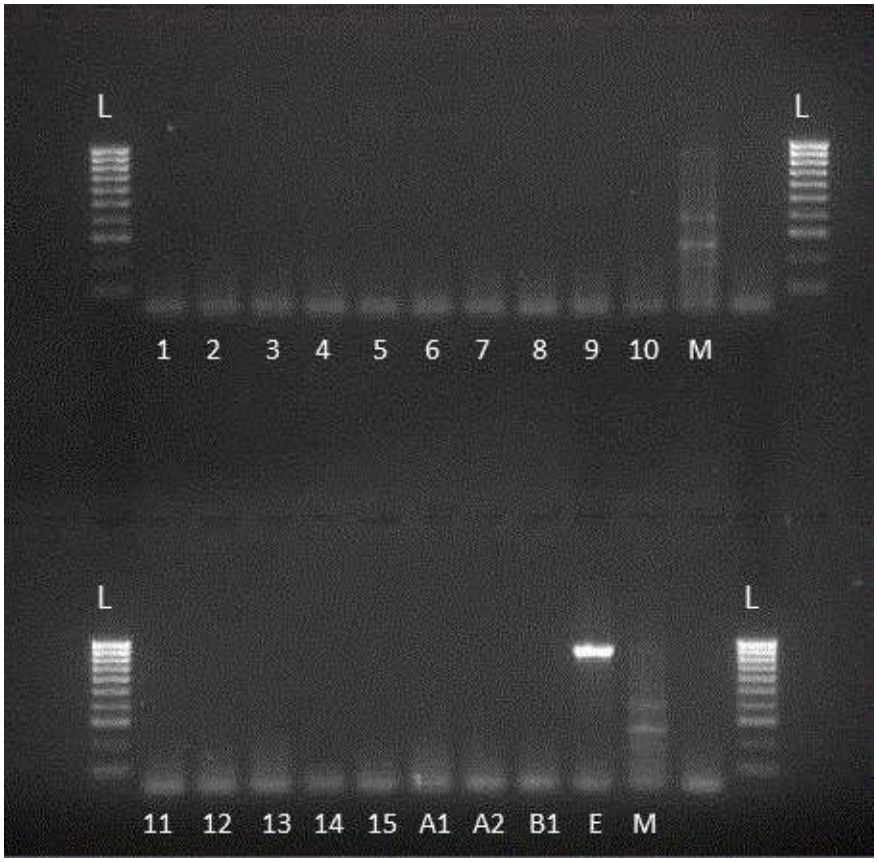


Fig A3: Agarose gel of PCR products, L= Ladder, numbers 1-15 represent samples from piezometers,A1-B1 streams, E cultured *E. coli* positive control and M positive control. Lanes un-labelled are negative controls.

References

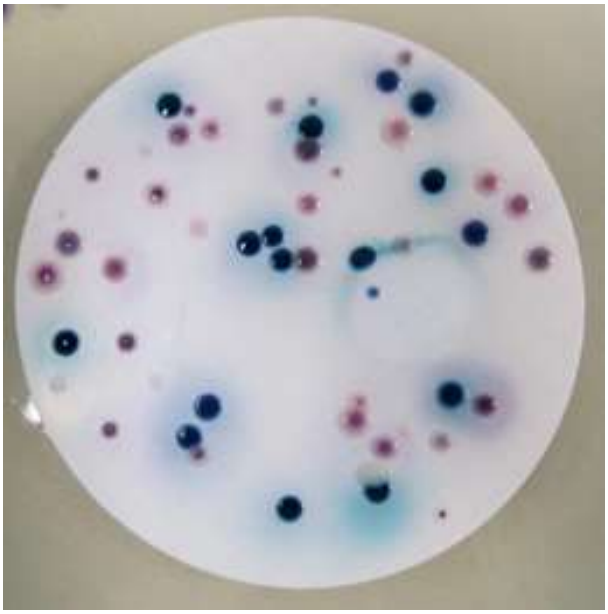
GRIFFITHS, R. I., WHITELEY, A. S., O'DONNELL, A. G. & BAILEY, M. J. 2000. Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. *Applied and Environmental Microbiology,* 66**,** 5488-5491.

APPENDIX VII: **Fieldwork and experimental images.**

Aberffraw flooded dune slack.

Aberffraw locating piezometer in a flooded dune slack.

Adding organic matter to nitrogen budget mesocosm experiment (Chapter 4 and 5).



Cultured *E. coli* (blue) and coliforms (purple) on Harlequin agar plate (Chapter 3).